JOURNAL OF PHARMACY AND PHARMACOLOGY

VOLUME XV No. 7 / JULY 1963

Published by Direction of the Council of

THE PHARMACEUTICAL SOCIETY OF GT. BRITAIN

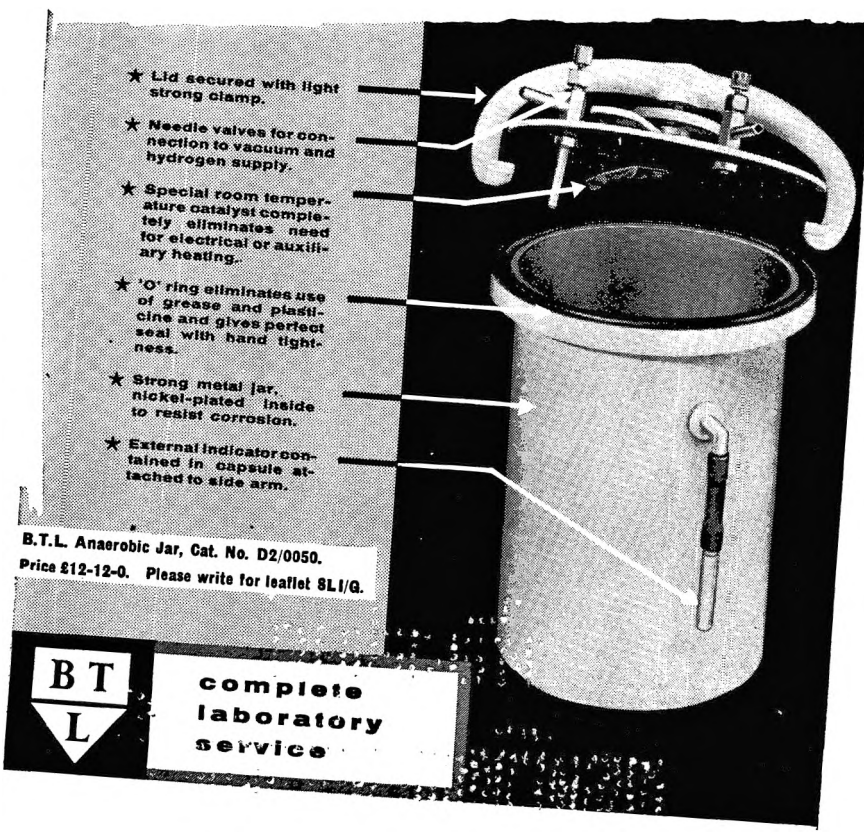
17 BLOOMSBURY SQUARE, LONDON, W.C.1

SOLD

to over 4,000 satisfied buyers, this most desirable residence for anaerobes. All mod cons.– including room temperature catalyst, all metal construction, unique "o" ring sealing.

by BAIRD & TATLOCK (LONDON) LTD., CHADWELL HEATH, ESSEX, ENGLAND

Branches in London, Manchester &,Glasgow. Agents throughout the U.K. and all over the world.



B.T.L. Anaerobic Jar, Cat. No. D2/0050.
Price £12-12-0. Please write for leaflet 8LI/G.

BTL complete laboratory service

TAS/BT108

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.
Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.
Annual Subscription **£5 0s. 0d.** Single Copies **10s.**
17 BLOOMSBURY SQUARE, LONDON, W.C.1
Cables: Pharmakon, London. W.C.1. *Telephone:* HOLborn 8967

Vol. XV No. 7 **July, 1963**

CONTENTS

RESEARCH PAPERS

A NEW TECHNIQUE FOR INVESTIGATING THE PROCESS OF TABLET COMPRESSION: A PRELIMINARY REPORT

By K. Marshall

From the Department of Pharmacy, Institute of Technology, Bradford, 7

Received February 13, 1963

This preliminary report covers static tests involving the incorporation of an electrical conducting material in a tablet formula and subsequent recording during compression of the change in electrical resistance of the compact. An assessment of the experimental variables has indicated optimum conditions for making dynamic tests on an instrumented tablet machine and suggested more refined adaptations of the technique. Comparisons between this electrical property of the material and other properties such as relative volume, initial granule size, moisture content of the granules and crushing strength of the compact are presented. The resistance plots show evidence of change in the physical condition of the material under pressure; that is an initial packing stage followed by a brief period during which the granular structure is capable of supporting the imposed load without breakdown and finally gradual failure of the granules to give a compact mass.

The behaviour of powdered metals during compression has been expressed in terms of the changing physical condition of the material by Seelig and Wulff (1946) who divided the process into three stages. (i) Packing of the particles. (ii) Elastic and plastic deformation. (iii) Cold working with or without fragmentation. Train (1956) showed that this sequence of events occurred in other powdered materials and Seth (1956) suggested that the formation of pharmaceutical tablets from granules might follow as a result of similar changes.

Jones (1960) referred briefly to the use of electrical conductivity measurements as a means of following the sintering process of powdered metals and Duffield and Grootenhuis (1958–59) described the method in some detail during their studies on the effect of particle size on the sintering of copper powder.

Changes in the properties of compressed materials have also been followed by means of electrical conductivity measurements on the compacts at various pressure levels. Shapiro (1948) used this technique during his investigations on the ageing of silver bromide precipitates and Huffine (1956) made similar experiments with sodium chloride and copper sulphate. In both these instances the authors measured the ionic conductivities of the salts and Huffine concluded that the method was of limited value because of the sensitivity of ionic conductance to certain properties of the specimen. In addition, these investigations were made under essentially static conditions such as are not encountered in the manufacture of tablets.

An attempt has been made to develop a technique which might subsequently yield useful information under operating conditions as near as

possible to those of the normal tabletting process. This report is an account of the initial work, carried out under static conditions, to determine the significance of certain experimental variables and the optimum conditions for translation to an instrumented tablet machine.

EXPERIMENTAL

Tablet granules were prepared incorporating a small amount of carbon in the form of synthetic graphite powder. The change in electrical resistance of the dried granular mass was determined at suitable intervals during its subsequent compression.

Preliminary experiments indicated the need for rigid control in the formulation and preparation of the granules, as well as in the methods of compression and recording of change in resistance. Standard techniques were therefore used for each of the above operations. Large variations were found in the conductivity of graphite obtained from different sources and the same batch of material was therefore used for all the experiments.

Formulation and Preparation of the Granules

Because of its known freedom from compression difficulties sodium chloride was chosen as the base material; this was mixed with the graphite in a small end-runner mill, both materials being in powder finer than 350 mesh. Half strength Mucilage of Acacia B.P.C. was then added as granulating fluid and binding agent and a further 5 min. allowed for mixing. The mass was then granulated by hand through a number 22 sieve and dried for 4 hr. at 60°.

The granules produced in this manner were separated into various size ranges by an Inclyno sieve shaker. Except where otherwise indicated the granules were stored over silica-gel in a desiccator for 48 hr. in order to achieve some uniformity of moisture content before tabletting.

A graphite content of 8 to 10 per cent was selected from tests which indicated that this concentration would give a range of resistance readings capable of application to a continuous recorder for use with the instrumented tablet machine. Lower concentrations of graphite gave very high resistance readings which were not reproducible.

Compression

A die fabricated from a non-conducting material was employed so that the electrical resistance of the compact could be measured during compression. From preliminary tests, Tufnol (a paper laminated synthetic resinoid material) was chosen as the most satisfactory material since it combined relative hardness with resilience and a surface which would take a reasonable polish. More recently polyacetal and polycarbonate resins have been tried and they may be equally suitable. A bakelite insert fitted within a metal die, as used by Huffine, was rejected in these experiments because of difficulties encountered when using such an assembly in a tablet machine. The punches used were standard $\frac{1}{4}$ in. flat faced punches from a Manesty hand tablet machine.

100 mg. of the granules was fed into the die and the punch and die assembly (Fig. 1) was placed between the platens of a hydraulic press. The electrical resistance between the faces of the two punches, at each pressure level, was determined using a Cambridge Decade Bridge. The experiments were carried out 10 times for each series of conditions and the experimental points recorded therefore represent the arithmetic mean of 10 readings. Most of the resistance readings, especially those at the high pressure end of the scale, showed little variation (less than 2 per cent). The variation however, was greater at low pressures. Some idea of this variation may be obtained from the standard deviation calculated from 30 measurements obtained at the lowest pressure; that is a pressure gauge reading of 20 p.s.i. The calculated value for the standard deviation was 2,725; the mean resistance reading at this pressure being 12,710 ohms. Despite this variation, the curves from individual sets of readings were always of the same general shape as those shown in the figures.

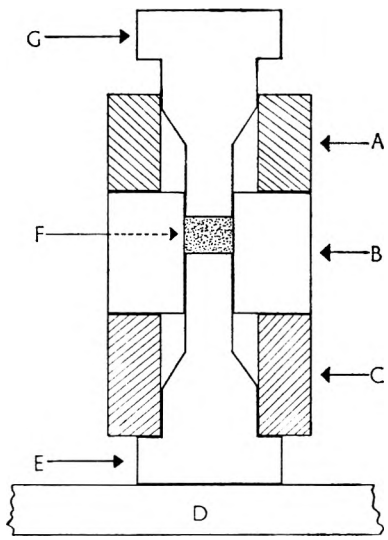


FIG. 1. Diagram of the punch and die assembly. A, Upper punch support. B, Tufnol die. C, Steel support for the die. D, Base plate (insulated from the press platform but carrying the connection to the decade bridge from the lower punch). E, Lower punch. F, Granules in the die. G, Upper punch (connected to the decade bridge).

A compact weight of 100 mg. was chosen as appropriate for a tablet diameter of $\frac{1}{4}$ in. and the applied pressures were selected to include the working range of the tablet machine. Resistance values at pressure gauge readings below 20 p.s.i. were not reproducible and are not therefore included; pressure gauge readings in excess of 600 p.s.i. frequently produced die failure. The pressure readings were obtained from a gauge connected to the main chamber of the hydraulic press and although this method does not indicate the absolute pressure transmitted to the compact it was thought sufficiently accurate for this preliminary study. It is

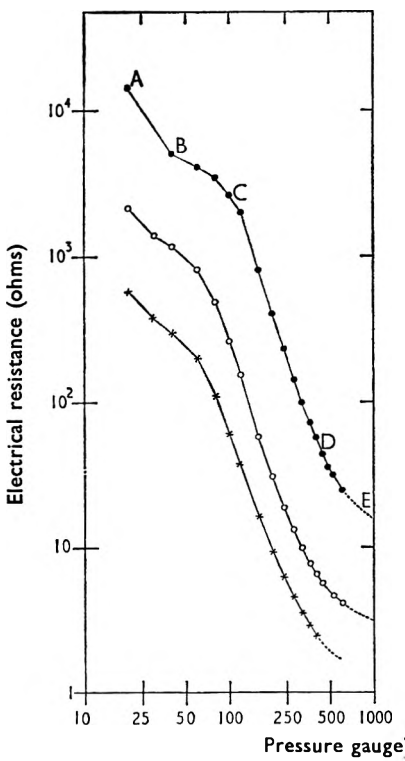
anticipated that strain gauges will be the method employed for determining pressures on the instrumented tablet machine thereby eliminating this approximation.

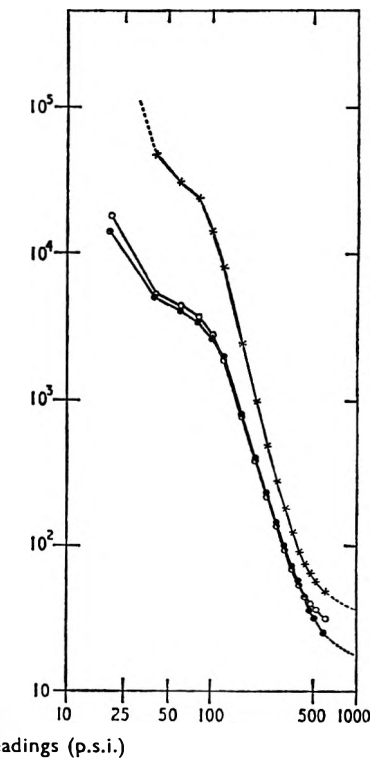
In certain of the tests the relative displacement of the two punches at each pressure level was measured using a Pye cathetometer so that the volume of the compact might be calculated.

Results

Fig. 2 shows the results of compressing 100 mg. of 25 on 30 mesh granules containing 8 per cent of graphite up to pressure gauge readings of 640 p.s.i. In the same figure the effect of variation in the graphite content is shown by the curves for granules containing 9 per cent and 10 per cent graphite.

Fig. 3 shows the effect of initial granule size on the resistance plot and Fig. 4 the effect of moisture content. The broken line portions of the





Pressure gauge readings (p.s.i.)

FIG. 2. The relation between compressional force and electrical resistance of a granular mass containing graphite.

8 per cent graphite —●—●—
9 per cent graphite —○—○—
10 per cent graphite —×—×—

FIG. 3. The relation between compressional force and electrical resistance of granules containing 8 per cent graphite showing the effect of variation in granule size.

25 on 30 mesh granules —●—●—
52 on 60 mesh granules —○—○—
120 on 240 mesh granules —×—×—

curves represent isolated readings taken with compressional force readings up to 1,000 p.s.i. It was impossible to obtain a full set of results in this region due to die failure at high pressures.

The crushing strength of compacts compressed to various pressure gauge readings was determined by means of a Strong-Cobb hardness tester and the results obtained are illustrated in Fig. 6.

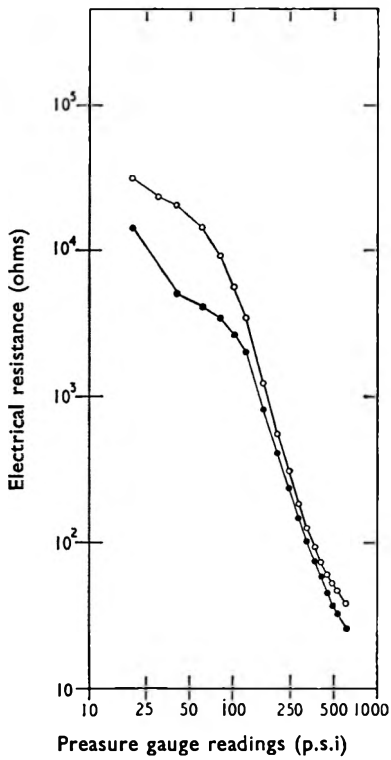


FIG. 4. The relation between compressional force and electrical resistance of granules containing 8 per cent graphite showing the effect of variation in the moisture content. Both sets of readings are for 25 on 30 mesh granules.

Air dry. —○—○—
Desiccated for 48 hr. —●—●—

DISCUSSION

The curves in Fig. 2 can be divided into sections. These, it is suggested, correspond to the stages in the change of the physical nature of a material under pressure referred to in the introduction. The findings of Shotton and Ganderton (1960) do not indicate a sharp demarcation between the various stages and this is in agreement with the results now reported.

It may be postulated that when pressure is first applied, the granules (which are loosely touching) move relative to one another to take up a

more compact arrangement and thereby increase the number of inter-granular contact points. Due to the applied pressure, the contacts will be of a more effective nature, i.e. they will possess a lower electrical resistance. It may be the improved inter-granular contact which chiefly

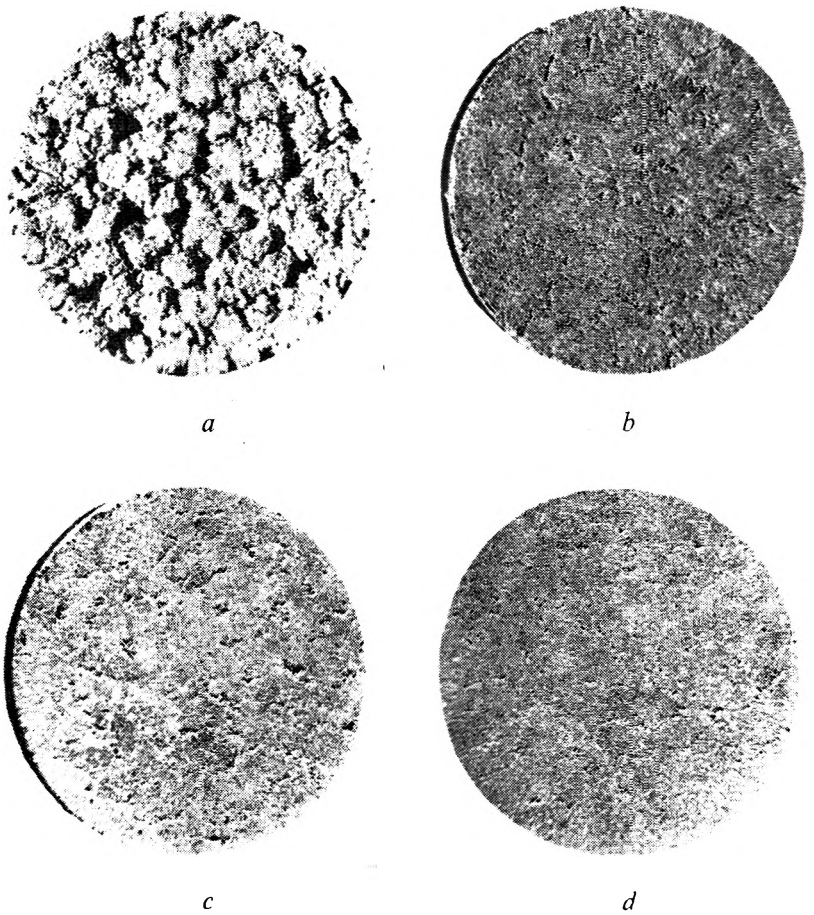


FIG. 5. The surface appearance of tablets after being subjected to the following compressional forces.

(*a*) 25 pounds per square inch.
(*b*) 100 „ „ „ „
(*c*) 200 „ „ „ „
(*d*) 400 „ „ „ „

all at a magnification of 7½ times.
Graphite content 8 per cent, granule size 25 on 30 mesh.

accounts for the marked fall in resistance (region A to B in Fig. 2). Train (1956) reported that at all stages of compression a skin was formed where the compacts had been in contact with the die walls. Though no

evidence of this was seen at low pressures in the present investigation, it is possible that the fall in resistance may be due to such a skin layer giving a preferential conducting path.

From B to C the granules are probably incapable of tighter packing without deformation but are strong enough to support the imposed load. The electrical resistance will therefore undergo only a small change due to further lowering of the inter-granular contact resistances and the onset of the third stage in localised areas of the compact. During stage 3, the granular structure fails, contact between the individual particles within the granules will therefore be improved and the intra-granular contact resistances will be reduced. This effect would account for the steady fall in electrical resistance in the region C to D in Fig. 2.

Finally with complete breakdown of the granular structure the maximum conducting effect of the graphite content will be approached and from point D onwards increase in pressure will produce less and less effect on the resistivity of the compact.

The above interpretation of the curves in Fig. 2 is supported by the changes in appearance of the tablet surface seen in Fig. 5 and by changes in the crushing strength of the compacts (Fig. 6). At pressure gauge readings up to about 25 p.s.i. the compacts possessed virtually no mechanical strength and the individual granules were clearly visible in a surface view of the tablet (Fig. 5a). Higuchi, Rao, Busse and Swintosky (1953) suggested an initial breakdown of granular structure, but it was found in the present work that granules retained their identity (i.e. could be recovered from the die apparently unchanged) up to pressure gauge readings of 20 p.s.i.

At pressure gauge readings between 100 and 500 p.s.i. the crushing strength of the tablet increased uniformly with increase in pressure and over this range the granular structure as shown by surface appearance (Fig. 5b, c and d) gradually disappeared. At pressure gauge readings above 500 p.s.i. the rate of increase in mechanical strength began to fall off and the surface of the tablet showed no trace of a granular structure.

The change in relative volume of the compacts with increasing pressure (Fig. 7) followed a similar curve to the resistance plot suggesting further evidence for the above argument. Relative volumes were calculated from the formula defined by Walker (1923) and used by Train (1956).

Although the results appear to favour this reasoning, further experimental work is desirable before the alternative explanation of preferential conduction via a skin layer can be excluded.

The curves showing the effect on the resistance of variation in the moisture content of the granules are confusing and several factors may be involved. The presence of moisture might be expected to affect the resistance readings irrespective of any action on the granule materials. Savage (1948) showed that while moisture is essential for graphite to act as an efficient lubricant, only a very low concentration was necessary. The lubrication effect is unlikely therefore to be a significant factor in the present work. It seems more feasible to suggest that, since the binding agent used was a gel, excessive desiccation might reduce its bonding

properties to such an extent that the granular structure failed more readily under compression. This would explain the lower resistance readings obtained with desiccated material. A more detailed study of this aspect of the problem with more accurate control and assessment of the moisture content is necessary.

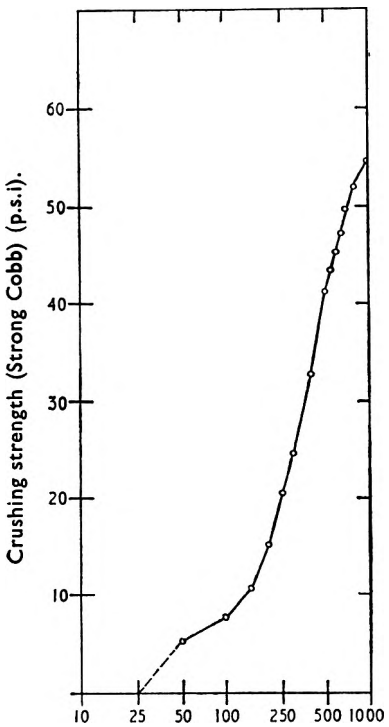


FIG. 6. The relationship between compressional force used and the crushing strength of tablets (Strong-Cobb). Graphite content 8 per cent, granule size 25 on 30 mesh.

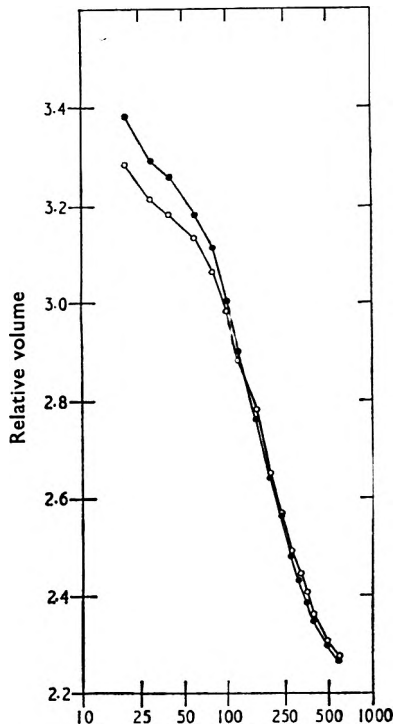


FIG. 7. The relationship between compressional force and the relative volume of tablets containing 8 per cent graphite.
25 on 30 mesh granules —●—●—
120 on 240 mesh granules —○—○—

It might be argued that the presence of graphite in the formulation would seriously affect the behaviour of the granular mass since it is known to possess marked lubricant properties. Train and Hersey (1960) have described experiments from which they conclude that the mechanism of lubrication by graphite and talc is somewhat akin to a roller-bearing action; they further deduce that under high compression this lubricant action is seriously curtailed.

To investigate the significance of the graphite in the present work, granules were prepared in which it was substituted by wood charcoal. Unfortunately a resistance range similar to that of graphite could only be

obtained with 100 per cent wood charcoal, that is, by omitting the sodium chloride completely. It was observed that these charcoal granules possessed very little mechanical strength and did not bond together to form a hard compact when compressed. In view of this it is not surprising that the resistance plot of the granules followed a curve similar to that obtained for 120 on 240 mesh granules of graphite (Fig. 2), though a strict comparison is hardly justifiable.

Granules containing powdered metals were also prepared but here again a higher concentration was found necessary before resistance readings within a useful range could be obtained. A further difficulty was chemical reaction at the metal surface which seriously impaired the conductivity. It is worth noting that, when powdered tin was used in a concentration of 40 per cent, the resistance readings remained above 1,000,000 ohms until the pressure readings reached a value of about 140 p.s.i.; at this point the resistance fell instantaneously to a value of 100 to 200 ohms and further increase in pressure soon reduced the value to less than one ohm. The most likely explanation for this phenomenon is that flow of the metal component occurred in addition to sudden breakdown of the granular structure. A similar result was recorded when compressing granules made from Mercury with Chalk B.P. 1948, though the sudden fall in resistance occurred at a lower pressure (approximately 50 p.s.i.). It would seem that graphite possesses a rather unique combination of properties which render it especially suitable for this type of investigation.

At this stage no attempt has been made to derive quantitative relationships between the variables involved in these experiments but it is anticipated that more refined techniques will offer an alternative method for obtaining information continuously during the normal operation of a tablet machine.

Acknowledgements. The author wishes to express his thanks to his colleagues at the Institute of Technology in Bradford and to Dr. R. C. Kaye for helpful discussion and advice during this work. The supply of information on and samples of the various materials used for the dies by Tufnol Ltd., Precision Products (Leeds) Ltd. and The Nylonic Engineering Co., Ltd., is also gratefully acknowledged.

REFERENCES

Duffield, A. and Grootenhuis, P. (1958–59). *J. Inst. Metals.*, **87**, 33–41.
Higuchi, T., Rao, A. N., Busse, L. W. and Swintosky, J. V. (1953). *J. Amer. pharm. Ass., Sci. Ed.*, **42**, 194–200.
Huffine, C. L. (1953). *Thesis for Doctorate of Philosophy*, Univ. of Columbia.
Jones, W. D. (1960). *Fundamental Principles of Powder Metallurgy*, p. 427. London: Edward Arnold.
Savage, R. H. (1948). *J. Appl. Phys.*, **19**, 1–10.
Seelig, R. P. and Wulff, J. (1946). *Trans. Amer. Inst. min. (metall) Engrs.*, **166**, 492.
Seth, P. L. (1956). *The Influence of Physical and Mechanical Factors in Tablet Making.* Calcutta. Published by the author.
Shapiro, I. and Kolthoff, I. M. (1948). *J. Phys. and Coll. Chem.*, **52**, 1319–1331.
Shotton, E. and Ganderton, D. (1960). *J. Pharm. Pharmacol.*, **12**, 87*T*–96*T*.
Train, D. (1956). *Ibid.*, **8**, 745–761.
Train, D. and Hersey, J. A. (1960). *Ibid.*, **12**, 97*T*–104*T*.
Walker, E. E. (1923). *Trans. Faraday Soc.*, **19**, 73, 83 and 614.

SURFACE-ACTIVE BETAINES: *N*-ALKYL-*NN*-DIMETHYLGLYCINES AND THEIR CRITICAL MICELLE CONCENTRATIONS

BY A. H. BECKETT AND R. J. WOODWARD

From the School of Pharmacy, Chelsea College of Science and Technology, Manresa Road, London, S.W.3

Received November 12, 1962

A series of surface-active *N*-alkyl *NN*-dimethyl glycines (alkyl betaines) and their hydrochlorides have been prepared. Some physical properties of these compounds have been investigated. The critical micelle concentrations of the betaines have been determined by a surface tension, a refractive index, a dye solubilisation and an iodine method. An explanation of the differences in critical micelle concentrations between these amphoteric surfactants and corresponding anionic and cationic surfactants is proposed.

AMPHOTERIC surfactants have not been studied so intensively as anionic, cationic or even non-ionic surfactants. Some amphoteric surfactants have been patented and a few marketed, e.g., Miranols (Mannheimer 1950), Ambiterics (Glovers Chemicals Ltd.) and Deriphats (General Mills). Incomplete details of the physical and solubilising properties have been obtained. Fundamental studies have not been possible because series of pure compounds of known chain length have not been available.

A series of pure non-ionic surfactants has recently been prepared in our laboratories (Mulley, 1960). We now report the preparation of a series of surface-active betaines which were required for detailed physical investigations.

Preparation of the Compounds

The alkyl betaines (*N*-alkyl *NN*-dimethylglycines) were prepared by treating a dimethylalkylamine with sodium chloroacetate (e.g., see Balle and Eisfeld, 1935; Tanaka, 1943). They were isolated from the sodium chloroacetate reaction mixture as their hydrochlorides (see Table II), which were then converted to the corresponding free bases by passing them through a suitable ion-exchange column. The eluate was evaporated to dryness and the residue recrystallised from ethanol : ether (1 : 20). The alkyl betaines (see Table III) were obtained as white amorphous solids.

Critical Micelle Concentrations

The critical micelle concentrations of the series of surfactants were determined by four independent methods; these were a surface tension, a refractive index, a dye solubilisation and an iodine method. The dye used was bromophenol blue and the iodine method was adapted from that used by Ross and Olivier (1959) for non-ionic surfactants.

EXPERIMENTAL

Synthesis

All melting points are uncorrected.

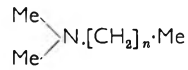
The alkyl bromides used were Eastman Kodak materials with a purity of 99 per cent.

The primary alkylamines used were 99 per cent pure.

Alkyldimethylamines. The tertiary amines were obtained by reaction of alkyl bromides with dimethylamine or by reductive methylation of a primary alkylamine with formic acid and formaldehyde (Kirby, 1941). The bases were purified by distillation. The quaternary iodide of each tertiary base was prepared by reaction of methyl iodide with the base dissolved in acetone. The details for each tertiary amine and its quaternary iodide are given in Table I.

TABLE I

NN-DIMETHYLALKYLAMINES AND THEIR QUATERNARY IODIDES



Alkyl chain		*NN*-Dimethylalkylamines			*NNN*-Trimethyl *N*-alkyl ammonium iodides						
		Distillation			Equiv. weight			Microanalytical data			
								Carbon		Hydrogen	
	n	Temp°	Pressure mm. Hg	Yield per cent	Calc.	Found	m.p.°C	Calc.	Found	Calc.	Found
Hexyl ..	5	143–4[a]	760	77	271	272	169[g]				
Octyl ..	7	79–80[b]	16	66	299	301	142[h]				
Decyl ..	9	62[c]	0·6	72	327	326	201–2[i]				
Undecyl ..	10	80	1·0	84	341	340	221–2	49·3	50·2	9·5	9·5
Dodecyl ..	11	95[d]	1·0	88	355	356	237–7·5	50·7	50·9	9·6	9·4
Tetradecyl	13	103–9[e]	0·5	72	383	382	238	53·3	53·6	10·0	9·7
Hexadecyl	15	142[f]	1·0	86	411	411	248–9[j]				

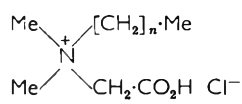
a-f. Gautier, Renault and Rabiant (1957) reported:

a. 145° at 760 mm. Hg
b. 189–190° at 760 mm Hg
c. 106° at 9 mm. Hg
d. 133° at 8 mm. Hg
e. 103–5° at 0·5 mm. Hg
f. 159–162° at 2·5 mm. Hg
g. Kato, Morikawa and Suzuki (1952) reported 166°
h. Kato and others (1952) reported 141°
i. Blomquist, Hallam and Josey (1959) reported 199–200°
j. Macovski (1936) reported 247°

N-*Alkyl* NN-*dimethylglycine hydrochlorides.* The alkyl-dimethylamine (1 equiv.) was refluxed in an ethanol:water mixture (1:1) with sodium chloroacetate (1 equiv.) until the system became monophasic.

TABLE II

N-ALKYL *NN*-DIMETHYLGLYCINE HYDROCHLORIDES



Alkyl chain		Yield per cent	Equiv. weight		m.p.°C.	Micro-analytical data					
						Carbon		Hydrogen		Nitrogen	
	n		Calc.	Found		Calc.	Found	Calc.	Found	Calc.	Found
Hexyl ..	5	51	224	224	156–7	53·7	54·2	9·9	9·9	6·3	6·2
Octyl ..	7	59	252	252	170–1	57·2	57·8	10·4	10·2	5·6	5·6
Decyl ..	9	71	280	280	172–3	60·1	59·9	10·8	10·7	5·0	4·8
Undecyl ..	10	74	294	294	170–1	61·3	61·3	11·0	11·0		
Dodecyl ..	11	54	308	306	165–6	62·4	61·8	11·1	10·8	4·5	4·4
Tetradecyl	13	53	336	336	163–4	64·4	64·2	11·4	11·3	4·2	4·0
Hexadecyl..	15	68	364	364	*a*	66·0	66·0	11·6	11·7	3·9	3·9

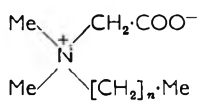
a. Melting point obscured by frothing of substance as it softened from 150° upwards.

The cooled reaction mixture was treated with excess concentrated hydrochloric acid. The hydrochloride crystallised and was filtered off, dried and recrystallised from ethanol : ether (2 : 1). In the case of the octyl and hexyl compounds, the acidified reaction mixture was evaporated to dryness and the hydrochloride was recrystallised after extracting the residue with hot ethanol. The details of these compounds are given in Table II.

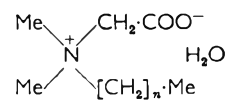
N-*Alkyl* NN-*dimethylglycines* (*Alkyl betaines*). The *N*-alkyl *NN*-dimethylglycine hydrochloride was dissolved in water and passed through an ion-exchange column of IRA-400 (OH). The eluate was evaporated to dryness. The final drying was carried out using xylene to form an azeotropic mixture with the water. The residue was recrystallised from ethanol : ether (1 : 20). The undecyl, decyl, octyl and hexyl compounds were dried by heating at their melting points in a vacuum. The details of these compounds are given in Table III.

TABLE III

N-ALKYL *NN*-DIMETHYLGLYCINES



I



II

Alkyl chain		Yield per cent	Equiv. weight		m.p.°C.	Micro-analytical data					
						Carbon		Hydrogen		Nitrogen	
	n		Calc.	Found		Calc.	Found	Calc.	Found	Calc.	Found
Hexyl[a] ..	5	89	187	188	138–40	64·1	62·8	11·3	11·1	7·5	7·4
Octyl[a] ..	7	93	215	216	155–6	66·9	67·2	11·7	11·8	6·5	6·3
Decyl[a] ..	9	100	243	244	162	69·1	68·4	12·0	12·2	5·7	5·7
Undecyl[a] ..	10	97	257	256	165	70·0	70·6	12·1	12·2	5·4	5·6
Dodecyl[b] ..	11	71	289	278	183	66·4	66·4	12·2	12·0	4·8	4·7
Tetradecyl[b]	13	92	317	300	198–9	68·1	68·3	12·4	12·3	4·4	4·5
Hexadecyl[b]	15	66	345	331	200–1[c]	69·5	69·1	12·5	12·5	4·1[d]	4·2[d]

a. Anhydrous alkyl betaine, as formula (I) above.
b. Hydrated alkyl betaine as formula (II) above.
c. Swain, Braun and Naegele (1953) reported 205–6°.
d. Swain and others (1953) reported nitrogen 3·97 per cent (calc. figure for anhydrous compound given as 4·29 per cent).

Equivalent weights of the bases were determined by titration with perchloric acid in glacial acetic acid using Oracet Blue as indicator. Mercuric acetate was added to the solution for the determinations on the hydrochlorides.

Critical Micelle Concentration Determinations

Materials. Iodine (Analar). Bromophenol Blue (Reagent Quality).

Apparatus. A Cambridge Du Nouy Tensiometer was used in the surface tension experiments.

The Hilger-Rayleigh Interferometer M.154 fitted with constant temperature water jacket was used for the refractive index measurements. A tungsten lamp and 1 cm. (or 10 cm. cells as indicated) were used.

A Hilger and Watts Uvispek Spectrophotometer was used to measure optical densities. 1 cm. silica cuvettes with water as reference solvent were used unless otherwise stated.

Surface tension method. The ring detachment method was used to determine the surface tension of the solutions and the Harkins and Jordan (1930) corrections were applied to the results. Measurements were made at 23 ± 1°. The results were plotted as surface tension against log surfactant concentration (e.g. see Fig. 1).

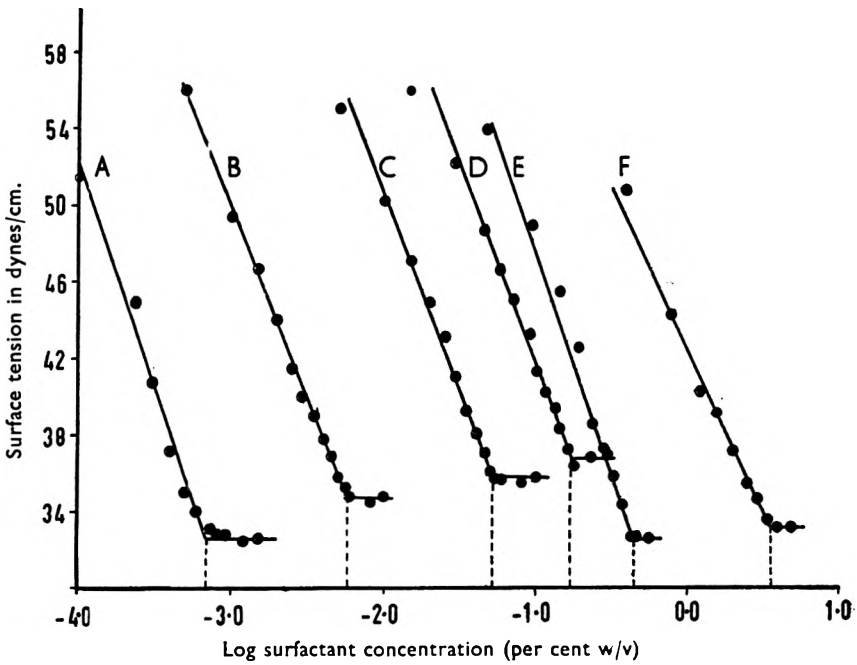


FIG. 1. Graphs of surface tension against log surfactant concentration used to determine the CMC of some alkyl betaines. A, Hexadecyl betaine. B, Tetradecyl betaine. C, Dodecyl betaine. D, Undecyl betaine. E, Decyl betaine. F, Octyl betaine.

*Refractive index method.** Measurements were made at 20·2 ± 0·1°. The first reading was made with the starting concentration in the two cells, and each solution of betaine was then used as reference solution for the next higher concentration of betaine; the differential refractive indices were summated. The results were plotted as changes in refractive index against molar concentration; the single breaks in the curves were taken as the critical micellar concentrations (CMC) (see Fig. 2).

Bromophenol Blue solubilisation method. A series of surfactant solutions in the CMC region were prepared in 1:250,000 aqueous Bromophenol Blue. The extinction of each solution was determined at 605 mμ. Measurements were made at 23 ± 1°. The results were plotted as extinction against surfactant concentration (e.g., see Fig. 3). The CMC values

* We thank N. Choulis for carrying out these measurements.

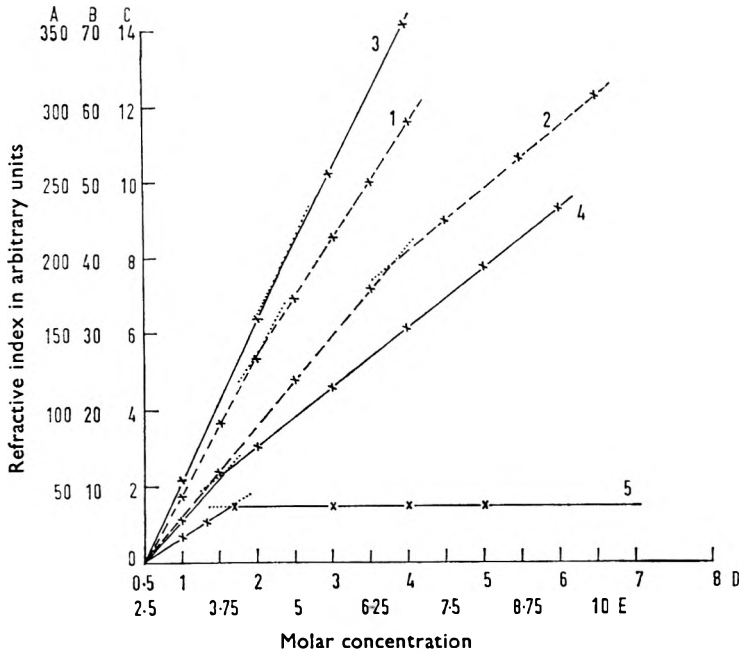


FIG. 2. Graphs of changes in refractive index against molar concentration in some betaines of structure $Me\cdot[CH_2]_n\cdot{}^+N\ Me_2\cdot CH_2COO^-$ using a 1 cm. (— — —) or 10 cm. (———) cell. 1, decyl A/D scale (10^{-2}M); 2, undecyl B/E scale (10^{-2}M); 3, dodecyl A/D scale (10^{-3}M); 4, tetradecyl B/D scale (10^{-4}M); 5, hexadecyl C/D scale (10^{-5}M).

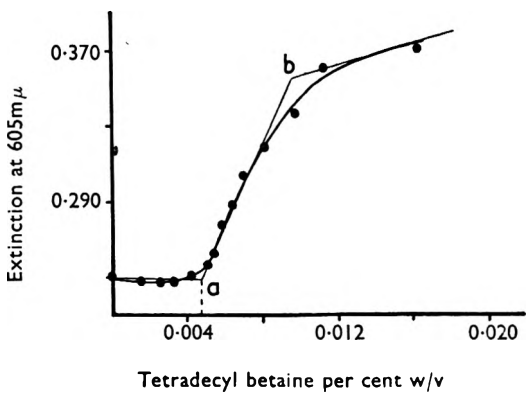


FIG. 3. Graph of optical density at 605mμ against tetradecyl betaine concentration per cent w/v plotted in determining the CMC by the solubilisation of bromophenol blue method. a, CMC = 0·0048 per cent w/v. b, Stable micelle point (Colichman 1951) where all dye is in the solubilised state.

of the decyl and octyl betaine were determined by titration of a solution of the surfactant, at a concentration above its CMC in aqueous Bromophenol Blue (1 : 250,000), with dye solution (1 : 250,000). The CMC was

calculated from the volume of the solution when the colour changed from blue to violet.

Iodine method. A solution of surfactant about 2–4 times the concentration of the CMC was prepared in aqueous iodine (15 mg./litre approx.). The solution was stored in the dark at 23 ± 1° for a week. It was diluted with water to give a series of solutions in the CMC region. The extinction of each solution was determined at 365 mμ. The results were plotted as extinction against surfactant concentration (e.g. see Fig. 4).

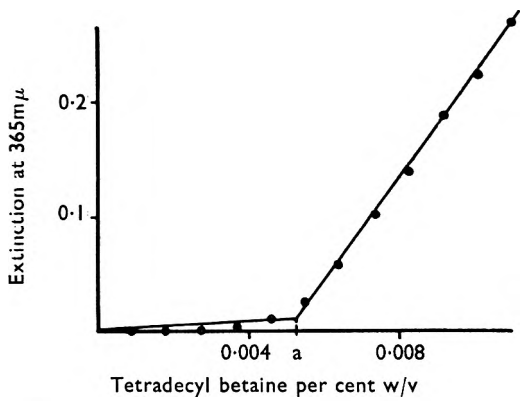


FIG. 4. Graph of observed optical density at 365mμ against tetradecyl betaine concentration plotted to determine the CMC by the iodine method. a = CMC = 0·0054 per cent.

RESULTS AND DISCUSSION

The *N*-alkyl *NN*-dimethylglycines with an alkyl chain greater than undecyl were obtained with a molecule of water of crystallisation. Betaines with an alkyl chain from hexyl to undecyl were obtained anhydrous provided they were subjected to severe drying conditions. The lower members of the series (hexyl and octyl betaines) were very deliquescent but this became less marked as the alkyl chain length was increased. The anhydrous compounds have sharp melting-points and end-points on titration as bases in non-aqueous media, but the hydrated compounds have indefinite values for these properties. The alkyl betaine hydrochlorides are white crystalline solids with sharp melting-points.

High purity of the present series of *N*-alkyl *NN*-dimethylglycines was indicated from the microanalytical data and the linear graphical relationship of alkyl chain length to log molar CMC. Microanalytical and physical data on the *N*-alkyl *NN*-dimethylglycine hydrochlorides indicated that these intermediates were pure.

Attempts to prepare the betaines from their hydrochlorides using inorganic bases gave products with appreciable inorganic impurity.

In the region of the CMC sharp changes occur in many of the properties of a surfactant solution. In the present work, methods for CMC determination other than those used successfully were tried. The zwitterion nature of the betaines was thought responsible for the unsuccessful

application of a conductivity method and complex polyphasic systems were obtained when attempting an octanol solubilisation method.

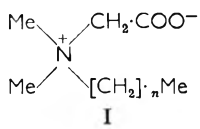
The results of the surface tension method for each alkyl betaine gave a graph on which the CMC was interpreted from the intercept of two straight lines (see Fig. 1). Below this value, the surface tension decreased linearly with increase in log surfactant concentration. Above it, the surface tension was almost constant. The results of the refractive index method gave graphs on which the CMC values were interpreted from the intercept of two straight lines (Fig. 2).

The results from the Bromophenol Blue solubilisation method gave a sigmoidal curve for each compound (e.g. see Fig. 3). This kind of curve has been obtained previously using the dye solubilisation methods (Mukerjee and Mysels, 1955; Colichman, 1951; Zutrauen, 1956). The CMC is usually interpreted from the lower surfactant concentration inflection (see Fig. 3).

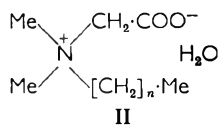
The iodine method was adapted from that used by Ross and Olivier (1959) for determining the CMC of non-ionic surfactants. The results for each determination gave two straight lines which intersected at the CMC (see Fig. 4). Extinction measurements at the absorption peak (365 mμ) on fresh aqueous surfactant with iodine systems were not reproducible hence, before use, the solutions were stored for a week in the dark. The variation in intensity at the absorption peak was attributed to chemical interactions.

TABLE IV

RESULTS OF THE CRITICAL MICELLE CONCENTRATION DETERMINATION ON A SERIES OF *N*-ALKYL *NN*-DIMETHYLGLYCINES



I



II

Alkyl chain		Critical micelle concentration in moles litre			
	n	Surface tension method	Dye solubilisation method	Iodine method	Refractive index method
Hexyl[a]	5	—	—	—	—
Octyl[a]	7	1.7×10^{-1}	1.7×10^{-1}	—	—
Decyl[a]	9	1.8×10^{-2}	1.8×10^{-2}	2.0×10^{-2}	2.1×10^{-2}
Undecyl[a] ..	10	6.6×10^{-3}	6.0×10^{-3}	6.4×10^{-3}	6.4×10^{-3}
Dodecyl[b] ..	11	1.8×10^{-3}	1.6×10^{-3}	1.8×10^{-3}	2.1×10^{-3}
Tetradecyl[b] ..	13	1.8×10^{-4}	1.5×10^{-4}	1.5×10^{-4}	1.7×10^{-4}
Hexadecyl[b] ..	15	2.0×10^{-5}	1.8×10^{-5}	1.8×10^{-5}	1.6×10^{-5}

a. Anhydrous alkyl betaine as formula (I) above.
b. Hydrated alkyl betaine as formula (II) above.
The critical micelle concentrations in moles/litre by the surface tension method of some compounds closely related to the alkyl betaines were found to be: dodecyl betaine HCl 1.98×10^{-3}; tetradecyl betaine HCl 1.96×10^{-4}; *NNN*-trimethyl *N*-dodecyl ammonium iodide 5.21×10^{-3}.

The four methods of determining the CMC of the alkyl betaines yielded similar results (see Table IV). This general agreement involving the binary systems (surfactant and water) using the surface tension and the refractive index method and those from the two types of ternary systems,

indicated that the addition of the stated amounts of either Bromophenol Blue or iodine did not appreciably affect the CMC.

The alkyl betaine surfactants are reported to be cationic (Moore, 1960) but behave differently from the quaternary ammonium surfactants. Moore (1960) suggested that the alkyl betaine surfactants should be classified as 'Intronium Surfactants' because they were not amphoteric. However, all quaternary amphoteric surfactants will fall into this class and it is probably less confusing if the amphoteric surfactants are divided under two headings i.e., the quaternary and the non-quaternary.

In the present work, the predominantly cationic nature of the alkyl betaines was indicated by the successful application of Bromophenol Blue, an anionic dye, for the CMC determinations. Attempted use of a cationic dye (Pinacyanol bromide) was unsuccessful. Corrin and Harkins (1947) found that a dye of opposite charge to the surfactant was essential in determining the CMC by a dye solubilisation method. The successful application of an iodine method indicated that the micelles of the alkyl betaines were not wholly negative or positive, since Ross and Olivier (1959) stated that iodine precipitated with positive micelles and did not interact with negative ones.

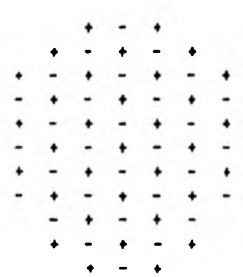


FIG. 5. Proposed arrangement of positive and negative charges on the surface of a micelle of an alkyl betaine

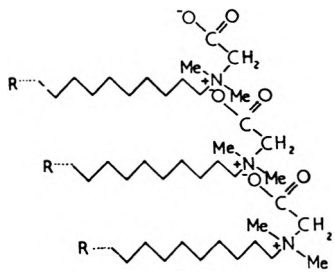
The surface of the amphoteric micelle is envisaged as covered with a net-work of positive and negative charges (see Fig. 5). The interior of the micelle in aqueous solution is assumed to be paraffinic in nature. In the present investigation, the alkyl betaines were dissolved in water and no foreign electrolytes were added. Thus, the balance between the charges on the micellar surface was dependent solely on the amphoteric character of the betaine molecule. The CMC depends on the lipophilic : hydrophilic balance in a surfactant molecule. Klevens (1953) proposed expression (I) for calculation of the CMC:

$$\log \text{CMC} = A - BN \quad .. \quad .. \quad .. \quad (1)$$

in which A is a constant depending on the homologous series of the surfactant, B is a constant which for most surfactants is equal to log 2 and N is the alkyl chain length. In anionic and cationic surfactants, A is the only function in (1) which is said to be dependent on the molecular structure and the term BN should be constant for all such surfactants with a straight

alkyl chain. Provided A is not the same for two series of such surfactants then the CMC values of corresponding members will be different but the change in CMC per carbon atom in the alkyl chain will be the same for both series.

The CMC values for a series of amphoteric surfactants have not been reported hitherto but these surfactants were said to have lower CMC values than corresponding anionic and cationic surfactants (Schmitz and Harris, 1958). This difference between such surfactants has been attributed to the desaturation of charges between the polar heads of amphoteric molecules, illustrated in the diagram III, leading to increased molecular



III

aggregation (McCutcheon, 1954). In the present series of alkyl betaines, not only was the position of the CMC different from corresponding anionic and cationic surfactants (related to A in expression 1), but so was the change in CMC with alkyl chain length (related to B in expression 1). The value for B in the series of alkyl betaines was found to be log 3 which indicated that changes in alkyl chain length had a greater effect on the CMC of these compounds than anionic or cationic surfactants. The polar heads of anionic or cationic surfactants repel one another but those of amphoteric agents have mutual attraction. Increase in alkyl chain length therefore has a greater effect on molecular aggregation when there is attraction rather than repulsion at the polar heads. Thus, the desaturation of charges (see III) is probably responsible for the lower CMC values and the greater effect of alkyl chain length on the CMC of the betaines than on corresponding anionic and cationic surfactants.

The alkyl betaine hydrochlorides showed similar CMC values and changes in CMC with alkyl chain length (see Table IV) to the free betaines. Thus the salts may be regarded as completely hydrolysed in aqueous solution.

The envisaged surface of the alkyl betaine micelle in aqueous solution (see Fig. 5) will provide a polarising surface for susceptible molecules.

Acknowledgement. R.J.W. gratefully acknowledges a Research Studentship from D.S.I.R.

REFERENCES

Balle, G. and Eisfeld, K. (1935). *U.S. Pat.*, 2,087,565.
Blomquist, A. T., Hallam, B. F. and Josey, A. D. (1959). *J. Amer. chem. Soc.*, **81**, 678–680.

Colichman, E. L. (1951). *Ibid.*, **73**, 3385–3388.
Corrin, M. L. and Harkins, W. D. (1947). *Ibid.*, **69**, 679–683.
Gautier, J. A., Renault, J. and Rabiant, J. (1957). *Bull. Soc. chim. Fr.*, 1014–1019.
Harkins, W. D. and Jordan, H. F. (1930). *J. Amer. chem. Soc.*, **52**, 1751–1772.
Kato, T., Morikawa, T. and Suzuki, Y. (1952). *J. pharm. Soc. Japan*, **72**, 1177–1182.
Kirby, J. E. (1941). *U.S. Pat.*, 2, 366, 534.
Klevens, H. B. (1953). *J. Amer. Oil Chem. Soc.*, **30**, 74–80.
Macovski, E. (1936). *Bull. Soc. chim.*, **3**, 498–500.
Mannheimer, H. S. (1950). *U.S. Pat.*, 2,528,378, 2,528,379 and 2,528,380.
McCutcheon, M. (1954). *Premier Congrès Mondial de la Détergence*, **3**, 1319–1322.
Moore, C. D. (1960). *J. Soc. cosmet. Chem.*, **11**, 13–25.
Mukerjee, P. and Mysels, K. J. (1955). *J. Amer. chem. Soc.*, **77**, 2937–2943.
Mulley, B. A. (1960). *Third International Congress of Surface Activity, Cologne*, **1**, 31–34.
Ross, S. and Olivier, J. P. (1959). *J. phys. Chem.*, **63**, 1671–1674.
Schmitz, I. A. and Harris, W. Sim. (1958). *Mfg. Chem.*, **29**, 51–54.
Swain, A. P., Braun, D. F. and Naegele, S. K. (1953). *J. org. Chem.*, **18**, 1087–1091.
Tanaka, F. (1943). *J. pharm. Soc. Japan*, **63**, 592–593.
Zutrauen, H. A. (1956). *J. Chim. phys.*, **53**, 62–70.

ASPECTS OF THE CLINICAL CHEMISTRY OF DESMETHYLIMIPRAMINE IN MAN

By Celia M. Yates, A. Todrick and A. C. Tait

From the Department of Clinical Research, Crichton Royal, Dumfries, Scotland

Received February 7, 1963

A method is described for the fluorimetric estimation of desmethylimipramine (DMI), an active metabolite of imipramine, in plasma. The plasma levels of DMI were followed in six patients over a period of 4 weeks during the treatment of endogenous depression. The results suggested that there was an inverse relation between the mean plasma DMI concentration and the weight of the patient. Two to 3 weeks treatment with DMI was necessary before the blood platelet 5-hydroxytryptamine was significantly lowered, whereas an improvement in symptoms, if it occurred, usually took place earlier.

Investigation into the biochemical actions of drugs used in the treatment of depression has received an impetus since the discovery that many antidepressant drugs inhibit monoamine oxidase. The rise in brain amines of animals after treatment with these drugs has been postulated to account for their therapeutic activity (Burns and Shore, 1961). Since it is impracticable to follow the levels of 5-hydroxytryptamine (5-HT) in human brain, the levels of 5-HT in the blood platelets have been measured in patients receiving antidepressant drugs. Those drugs which inhibit monoamine oxidase cause an increase in platelet 5-HT (Pletscher and Bernstein, 1958) whereas imipramine (Marshall, Stirling, Tait and Todrick, 1960), which has no inhibitory action on monoamine oxidase, causes a fall in platelet 5-HT.

The fact that only a proportion of depressed patients respond to treatment with antidepressant drugs could be due to individual failure of absorption. An estimate of the amount of drug in the plasma is therefore desirable before attempting to correlate behavioural response with biochemical effects.

Clinical investigation of desmethylimipramine (DMI) has followed the observation of Gillette, Dingell, Sulser, Kuntzman and Brodie (1961) that imipramine owes its activity to this metabolite. Brodie, Dick, Kielholz, Poldinger and Theobald (1961) and Meduna, Abood and Biel (1961) showed that DMI produced improvement in the mood of some depressed patients after only 2 days treatment, in contrast to imipramine which may take 2 to 3 weeks to produce any effect; on the other hand Oltman and Friedman (1962) found that DMI required 7–10 days to exert its therapeutic action.

We report the first stage of an attempt to correlate the behavioural effects of DMI with the level of the drug in the plasma and of 5-HT in blood platelets.

Methods

Blood was collected and a saline suspension of the platelets obtained by differential centrifugation by the method of Marshall, Stirling, Tait and

Todrick (1960). Platelets were counted by the method of Dacie (1956). The plasma, containing anticoagulant solution, was collected separately and both plasma and platelet suspension were stored at −15°.

Estimation of Desmethylimipramine

DMI differs chemically from imipramine only by the absence of one methyl group from the terminal nitrogen atom. Gillette, Dingell and Quinn (1960) have described a method for the estimation of imipramine in human plasma and found concentrations of 0·1–0·6 μg./ml. in patients receiving 150–300 mg. daily; they noted losses due to adsorption on glass in the heptane extraction procedure which they used (Quinn, personal communication). A protein precipitation procedure has therefore been tried in an attempt to avoid this difficulty. It was soon found that tryptophan, present in plasma to the extent of 10 μg./ml. (Duggan and Udenfriend, 1956) and fluorescing strongly with a peak at 280/360 mμ (uncorrected instrument reading), was liable to interfere, and subsequently preliminary investigations were made with DMI solutions containing tryptophan at the expected level. It was observed that the tryptophan fluorescence was high at neutral pH but much less in strong alkali, whereas the DMI fluorescence was lowest in acid solution and increased progressively with rise in pH. The following procedure was therefore adopted.

The plasma is diluted 1 in 5 with normal saline and 1·0 ml. is mixed with saline (1·0 ml.) or standard DMI in saline (1·0 ml.) in a 3 ml. centrifuge tube. 5 per cent zinc sulphate heptahydrate (0·4 ml.) is added followed by 0·3N barium hydroxide (0·3 ml.) (sodium hydroxide is not satisfactory; see Somogyi, 1945) with immediate mixing by inversion (6–8 times). After standing 20 min. in a refrigerator, the tube is centrifuged for 20 min. at 3,000 r.p.m. in an iced bucket (see Marshall and others, 1960).

Exactly 1 ml. of the supernatant is transferred to a quartz cuvette and the fluorescence measured in a spectrophotofluorimeter (Aminco-Bowman), at a setting of 280/360 mμ without altering the pH and at a setting of 280/440 mμ after the addition of 0·25 ml. 5N sodium hydroxide. Standards and blanks are run concurrently using plasma obtained from the same patient on the two days preceding the commencement of therapy.

The net fluorescence due to DMI or its metabolites is obtained by deducting the reagent blank and the residual fluorescence due to tryptophan. The ratio of the fluorescence due to tryptophan in alkali at 280/440 mμ to that in neutral solution at 280/360 mμ, determined in the presence of the reagents, was found to be 0·029.

Estimation of 5-Hydroxytryptamine

The protein precipitation procedure of Weissbach, Waalkes and Udenfriend (1958) developed for estimations in whole blood is applied to the platelet suspension; 10 per cent zinc sulphate monohydrate (0·3 ml.) and 0·42N sodium hydroxide (0·3 ml.) are added to a 2 ml. aliquot of the saline suspension of platelets, the tube being shaken after each addition. After 20 min. in a refrigerator the tube is centrifuged for 20 min. at 3,000 r.p.m. in an iced bucket. Exactly 1 ml. of the supernatant is

transferred to a quartz cuvette and the fluorescence measured in a spectrophotofluorimeter (Aminco-Bowman) set at 300/550 mμ both before and after acidification with concentrated hydrochloric acid (sp. gr. 1·18; 0·3 ml.).

Acidification has been found to quench the blank fluorescence partially and the net fluorescence due to 5-HT has been taken as "fluorescence in acid solution $-0{\cdot}7 \times$ fluorescence in neutral solution" for both unknown and standard solutions. Two standards are run with each batch (0·4 μg./ml. 5-HT creatinine sulphate = 0·174 μg./ml. free base) and the duplicates from each platelet suspension are run in different batches.

This method (Crosti and Lucchelli, 1962; Todrick, 1962) gives results agreeing with those obtained by the extraction procedure of Brodie, Tomich, Kuntzman and Shore (1957). However, owing to the omission of the extraction procedure, interference with the 5-HT estimation by the drug being studied is more liable to occur.

DMI being less fluorescent in acid than in neutral solution would, if present in effective concentration, produce a high blank reading but a negligible alteration in the fluorescence due to 5-HT after acidification; it would therefore cause a fictitiously low 5-HT estimate. The mean blank reading for pre-treatment bloods was 0·037 in arbitrary units (23 estimations); for bloods taken during treatment the mean blank was 0·034 (72 estimates); this indicates that interference has not occurred.

The effect of DMI on the estimation of 5-HT in aqueous mixtures subjected to the precipitation procedure has been checked. For DMI concentrations of 10, 1, 0·1 μg./ml., the estimation of 5-HT expressed as a percentage of the 5-HT found in the absence of DMI, gave 44, 93, 97 per cent respectively. Thus the 1 μg./ml. concentration of DMI will affect the estimation of 5-HT. However, the platelet button containing adhering plasma occupies a volume of approximately 0·1 ml. before being diluted to 5 ml. with saline. This means that the plasma DMI is diluted 50-fold before the 5-HT is estimated. The only way in which DMI could be present in amounts sufficient to interfere would be if it were concentrated in the platelets (as is 5-HT). Long and Lessin (1962) investigated this possibility with a series of drugs including imipramine and found no evidence of uptake against a concentration gradient.

RESULTS

Six female patients recently admitted to one ward of the hospital formed the group studied. The mean time interval between admission and commencement of therapy was 9 days. All six patients suffered from symptoms of depression and not all were first admissions. They received DMI (Pertofran: Geigy) orally in tablet form. The dosage schedule was 75 mg. for the first 3 days (in three doses at 9 a.m., 2 p.m. and 6 p.m.), 100 mg. on the fourth day, 125 mg. on the fifth day and 150 mg. on the sixth and subsequent days. Blood samples were taken on 4 or 5 days in the first week and thereafter at 7-day intervals. Blood was also collected from three controls (staff) not receiving the drug.

Effect of Acetylsalicylic Acid Preparations on DMI *Estimation*

After the observation of an unexpectedly high level of the drug in patient No. 5 at the end of the third week, it was discovered that this patient had received codeine compound tablets, containing 24 grains of acetylsalicylic acid in the 24 hr. before sampling. This compound fluoresces strongly in alkaline solution (peak 320/410 mμ uncorrected instrument reading). Two previous high figures, one in a patient and one in a control, which had been discounted as anomalies arising from a new technique, were scrutinised. Patient 2 had started on acetylsalicylic acid for rheumatoid arthritis a few days previous to the high level although the duration of therapy was not known. Control A admitted that he had probably taken the drug 1 or 2 hr. before collection of the sample showing an apparent high level of DMI. The apparent DMI concentrations (μg./ml. plasma) from two controls who received 10 grains of acetylsalicylic acid were: 25·3, 37·5, 10·7, 0·09 for subject A and 19·8, 39·5, 16·2 and 0·02 for subject D at ½, 2, 7, 24 hr. after dosage.

TABLE I

PLASMA DMI (μG./ML.) IN PATIENTS ON THERAPY AND IN CONTROLS

		Patients						Controls		
Day	Dose (mg.)*	1	2¶	3	4	5	6	A	B	C
1	75	0·08	—	—	0·08	0·15	0·20	0·10	0·02	−0·02
2	75	0·35	0·22	—	0·02	0·19	0·32	−0·02	0·04	−0·10
3	75	0·23	—	—	0·28	0·19	—	(0·44)‡	−0·19	—
3 or 4†	100	—	0·14	—	0·50	—	0·36 0·30	(79·0)‡	—	−0·03
5	125	0·40	0·89	—	—	0·75	—	—	0·23	−0·10
6–8	150	—	—	—	0·74	0·54	0·56	—	0·03	−0·09
13–15	150	0·83	1·24	—	0·79	(43·6)‡	0·70	—	0·03	0·06
20–22	150	0·82	(13·0)‡	0·86	1·34	0·53	0·79	0·05	(0·65)§	0·03
27–29	150	0·70	1·51	0·66	0·82	0·69	—	0·06	0·06	—
Mean at 150 mg.‖		0·78	1·38	0·76	0·92	0·59	0·68	0·05	0·03	−0·04
Clinical response at 14–21 days		Moderate	Slight	Moderate	Slight	Moderate	Moderate			
Bodyweight (kg.)**		79·4	46·7	56·0	52·2	63·6	55·3			

Footnotes:

* The dose refers to the total amount of DMI taken in the 24 hr. immediately before sampling.

† Due to blood being collected from some patients regularly at 9 a.m. and from others at 2 p.m. the dose on the 24 hr. preceding the third day could be either 75 or 100 mg.

‡ Subject known to have been taking acetylsalicylic acid about this time (see text).

§ Subject has no recollection of having taken acetylsalicylic acid at this time.

‖ Omitting all figures in brackets.

¶ Samples after 150 mg. from this patient were withdrawn 3 days earlier than stated under "day."

** Weights were obtained retrospectively from the ward records; they include clothing.

Relation of DMI *Plasma Levels to other Factors*

Plasma levels of DMI in patients and controls are given in Table I. Mean values for the group at different dose levels are plotted in Fig. 1. After reaching the maximum (150 mg.) daily dose, the levels for a given patient remain fairly constant for three weeks. The range of 0·59 μg./ml. to 1·38 μg./ml. for the group is not excessively wide. The number of cases is rather small to expect any significant correlation with clinical

results. In fact, on simple clinical assessment patients 2 and 4 responded less well than the others; their mean plasma DMI levels at 150 mg. were the highest in the series. Ordinarily, some degree of improvement—largely represented by an increase in psychomotor activity—was evident at one week and a noticeable therapeutic effect by 10–14 days.

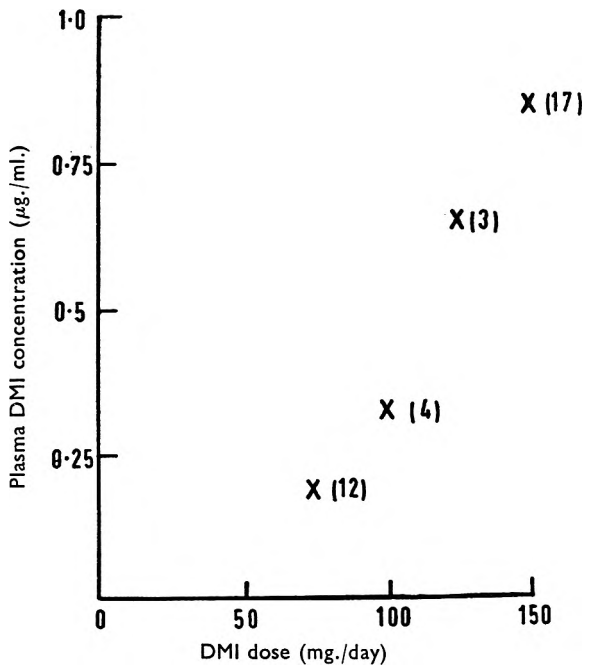


FIG. 1. Effect of dosage level on DMI plasma concentration. Figures in brackets are number of estimates on which value is based.

There is however a strong suggestion of an inverse relation between the mean final plasma concentration and body weight. The correlation coefficient $t_{10} = -0{\cdot}55$ (for $P = 0{\cdot}05$, $t_{10} = -0{\cdot}57$).

Platelet 5-HT *Levels in Patients Receiving* DMI

The physiological factors affecting platelet 5-HT level are not well understood and considerable day-to-day variation sometimes occurs in individuals even when they are not receiving drugs known to affect 5-HT metabolism. In one patient (No. 4) the pre-treatment levels were less than half the lower limit of normality and all data from her have been omitted. The remainder had normal pre-treatment levels (Todrick, Tait and Marshall, 1960) with a mean of 0·18 μg./ml. of whole blood.

Data from each individual have been converted to percentages of her mean pre-treatment level and these subsequently grouped. The change in 5-HT level during therapy is shown in Fig. 2. The length of the bar on each side of a point is the product of the standard error of the mean and t for $P = 0{\cdot}05$ for the appropriate number of degrees of freedom.

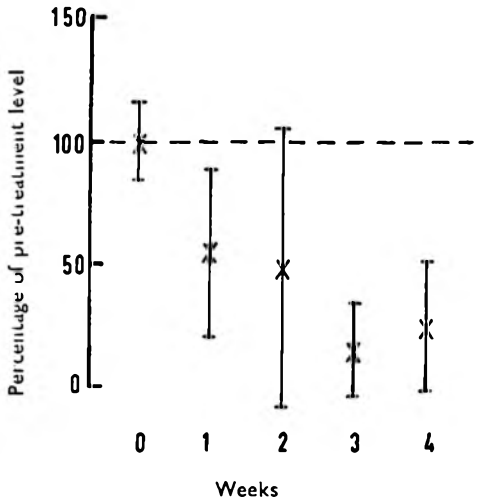


FIG. 2. Effect of DMI on blood platelet 5-HT level.

DISCUSSION

It has been shown that the antidepressant drug imipramine is metabolised in the body both by successive demethylation of the nitrogen atom on the side chain, and by hydroxylation followed by glucuronide formation; a compound involving both pathways has also been isolated from the urine (Herrmann, Schindler and Pulver, 1959; Herrmann and Pulver, 1960).

It has been suggested (Gillette and others, 1961) that the antidepressant action is due to the first product of demethylation, desmethylimipramine, the subject of this paper, and that this substance produces a more rapid therapeutic effect.

The fluorescence spectra and quantum efficiencies of imipramine and desmethylimipramine are so similar that the estimation of these individually in a mixture would involve considerable difficulty. The choice of desmethylimipramine for this study of the correlation between biochemical and behavioural response to antidepressant medication was made partly with a view to avoiding this difficulty; however, it now appears that the metabolite produced by the further demethylation of DMI also possesses the same fluorescence characteristics, though the hydroxylated metabolite does not (Haydu, Dhrymiotis and Quinn, 1962); the plasma levels quoted therefore refer to total DMI and its demethylated metabolite.

From the point of view of the hypothesis that differential clinical response is due to failure in absorption this is not so critical. The plasma content of DMI is a small fraction of the total present in the body (Herrmann and Pulver, 1960) but it is reasonable to assume and the data suggest that after 2–3 weeks on a fixed dosage schedule equilibrium conditions exist. The concentration range 0·59–1·38 μg./ml. observed does not indicate any major failure of absorption in any individuals. Indeed if the apparent

inverse relation between plasma DMI concentration and body weight should be confirmed this would mean that the actual amount absorbed would vary even less between patients than the individual plasma levels suggest. The hypothesis is therefore not supported by the evidence so far presented.

Haydu and others (1962) have recently reported briefly on a similar investigation made with imipramine. They observed a significantly higher mean plasma "iminodibenzyl" level in four patients who failed to respond. Our clinical observations do not contradict this unexpected conclusion.

The reduction in platelet 5-HT level which occurs during DMI therapy appears to follow much the same time course as that caused by imipramine (Marshall and others, 1960). The relationship of this to the clinical response is a matter of uncertainty since Kivalo, Rinne and Karinkanta (1961) and Schanberg and Giarman (1962) have observed that imipramine causes a slight rise in the brain 5-HT levels of rats, though much less than that caused by amine oxidase inhibitors. Himwich, Costa and Himwich (1961) have however found in dogs a fall in free 5-HT coupled with a rise in bound 5-HT. There was little overall change in the amount of 5-HT in the hippocampus, pons and midbrain, but a fall in the amygdala and a rise in the caudate nucleus.

Acknowledgements. This work was supported by a grant made to one of us (A.C.T.) by the Scottish Hospital Endowments Research Trust. We are indebted to Miss Aileen B. McLaren for valuable technical assistance; to Drs. H. Ferguson and D. E. Wallace for clinical assessments; and to Dr. T. E. Grant, Geigy Pharmaceutical Company Ltd. for his helpful co-operation.

REFERENCES

Brodie, B. B., Dick, P., Kielholz, P., Poldinger, W. and Theobald, W. (1961). *Psychopharmacologia*, **2**, 467–474.
Brodie, B. B., Tomich, E. G., Kuntzman, R. and Shore, P. A. (1957). *J. Pharmacol.*, **119**, 461–467.
Burns, J. J. and Shore, P. A. (1961). *Ann. Rev. Pharmacol.*, **1**, 79–104.
Crosti, P. F. and Lucchelli, P. E. (1962). *J. clin. Path.*, **15**, 191–193.
Dacie, J. N. (1956). *Practical Haematology*, 2nd ed., p. 49, London: J. and A. Churchill Ltd.
Duggan, D. E. and Udenfriend, S. (1956). *J. biol. Chem.*, **223**, 313–319.
Gillette, J. R., Dingell, J. N. and Quinn, G. P. (1960). *Fed. Proc.*, **19**, 137.
Gillette, J. R., Dingell, J. N., Sulser, F., Kuntzman, R. and Brodie, B. B. (1961). *Experientia*, **17**, 417–418.
Haydu, G. G., Dhrymiotis, A. and Quinn, G. P. (1962). *Amer. J. Psychiat.*, **119**, 574–575.
Herrmann, B. and Pulver, R. (1960). *Arch. int. Pharmacodyn.*, **126**, 454–469.
Herrmann, B., Schindler, W. and Pulver, R. (1959). *Med. exp.*, **1**, 381–385.
Himwich, W. A., Costa, E. and Himwich, H. E. (1961). *Proceedings of the Second Meeting of the Collegium Internationale Neuropsychopharmacologium*, pp. 485–489, Editor Rothlin, E., Amsterdam: Elsevier.
Kivalo, E., Rinne, U. K. and Karinkanta, H. (1961). *J. Neurochem.*, **8**, 105–108.
Long, R. F. and Lessin, A. W. (1962). *Biochem. J.*, **82**, 4P.
Marshall, E. F., Stirling, G. S., Tait, A. C. and Todrick, A. (1960). *Brit. J. Pharmacol.*, **15**, 35–41.
Meduna, L. J., Abood, L. G. and Biel, J. H. (1961). *J. Neuropsych.*, **2**, 232–237.
Oltman, J. E. and Friedmann, S. (1962). *Amer. J. Psychiat.*, **119**, 370–371.
Pletscher, A. and Bernstein, A. (1958). *Nature (Lond.)*, **181**, 1133–1134.

Schanberg, S. M. and Giarman, N. J. (1962). *Biochem. Pharmacol.*, **11**, 187–194.
Somogyi, M. (1945). *J. biol. Chem.*, **160**, 69–73.
Todrick, A. (1962). *Proc. Ass. clin. Biochem.*, **2**, 65–66.
Todrick, A., Tait, A. C. and Marshall, E. F. (1960). *J. ment. Sci.*, **106**, 884–890.
Weissbach, H., Waalkes, T. P. and Udenfriend, S. (1958). *J. biol. Chem.*, **230**, 865–871.

DETERMINATION OF BLOOD AND OTHER TISSUE CONCENTRATIONS OF PARACETAMOL IN DOG AND MAN

J. R. GWILT,* A. ROBERTSON* AND E. W. MCCHESNEY†

*From the *Winthrop Laboratories, Newcastle upon Tyne 3 and †Sterling-Winthrop Research Institute, Rensselaer, N.Y.*

Received March 15, 1963

The even distribution of non-conjugated paracetamol in the tissue waters of the dog has been confirmed. The mean tissue water: plasma water concentration is 1·1. A technique for the determination of non-conjugated paracetamol in man has been devised from existing methods. It has increased sensitivity and is applicable to the routine determination of a large number of samples. This method determines only non-conjugated paracetamol, the sulphated and glucuronated conjugates do not interfere with its accuracy, and it gives results comparable with previous methods. In man, 45 min. after oral administration of paracetamol, the whole blood: plasma concentration ratio is virtually constant at 1·1, indicating that the determination of non-conjugated paracetamol in whole blood should give a satisfactory indication of tissue level.

THE only detailed study which appears to have been made on the distribution of paracetamol (*N*-acetyl-*p*-aminophenol) in tissues (Brodie and Axelrod, 1949) was done using one dog given phenacetin by mouth. Paracetamol levels were determined because these workers had previously shown it to be the major metabolite of phenacetin. Subsequently, Clark (1951) demonstrated that the metabolic pathways of paracetamol in man and the dog were similar. Because of the limited evidence available we have repeated the work of Brodie and Axelrod on the tissue distribution of paracetamol in the dog.

The original analytical method of Brodie and Axelrod was used. A modification of this was subsequently used for the estimation of blood concentrations in man.

EXPERIMENTAL METHODS

Tissue Studies of Paracetamol in Dogs

Five dogs were given 300 mg./kg. paracetamol by mouth. After 2 hr. they were killed by intravenous pentobarbitone. The dose and time were chosen so that the tissue concentrations of the drug could be compared directly with those of Brodie and Axelrod (1949) for non-conjugated paracetamol values after administration of an equivalent dose of phenacetin.

Determination of tissue levels was made by the method of Brodie and Axelrod (1948, 1949), except that the pH for extraction was changed because it gave unsatisfactory results for liver homogenates. Tissues were homogenised in 0·1N hydrochloric acid, and the homogenate neutralised and buffered at pH 6·6, before the extraction of non-conjugated paracetamol. By adding known amounts of the drug to tissues from treated animals, recovery was found to be 86 ± 3 per cent from liver

homogenate to which 6–96 mg./kg. had been added and 96 ± 4·5 per cent for muscle homogenate to which 6–96 mg./kg. had been added.

Results from the tissues of the treated animals are in Table I. The figures have also been calculated to give the proportion of paracetamol in tissue water related to its concentration in plasma water—assuming that water represents 92 per cent of the total plasma, and that the tissue water contents per cent are: liver 72, kidney 77, heart 76, spleen 75, lung 76 and muscle 73. These figures were also used by Brodie and Axelrod (1949).

Analytical Techniques in Man

Because the method of Brodie and Axelrod (1949) was found to be unsuitable for large numbers of samples, a method was devised which was a combination of the procedures of Lester and Greenberg (1947) and of Brodie and Axelrod (1948). The detailed method is described below. Essentially, whole blood is triturated with sodium sulphate to give a dry friable mass from which free paracetamol can be extracted with ether.

TABLE I

TISSUE LEVELS OF PARACETAMOL IN DOGS 2 HR. AFTER AN ORAL DOSE OF 300 MG./KG.
(Values given as mg./kg. net weight of tissues)

Tissue	Dog, sex and wt. (kg.) A F, 8·5	B M, 11·2	C M, 11·7	D M, 10·1	E M, 10·7	Mean ± s.e.	Brodie and Axelrod‡
Plasma	169	156	103	123	147	140 ± 12	96
Liver*	210	130	120	112	149	144 ± 18	99
Kidney	228	159	108	116	136	149 ± 22	104
Heart	204	132	92	113	133	135 ± 19	79
Spleen	178	109	86	96	115	117 ± 16	80
Lung	182	114	99	101	127	125 ± 15	88
Brain§	176	135	83	87	140	124 ± 17	82
Muscle	179	139	95	99	146	132 ± 16	69
Fat§	24	21	13	14	18	18 ± 2	—
	Tissue water : plasma water concentration ratios						
Liver	1·59	1·06	1·51	1·15	1·29	1·32 ± 0·10	1·32
Kidney	1·62	1·21	1·25	1·12	1·10	1·26 ± 0·09	1·29
Heart	1·47	1·02	1·08	1·11	1·09	1·15 ± 0·08	1·00
Spleen	1·29	0·85	1·02	0·95	0·96	1·01 ± 0·07	1·00
Lung	1·30	0·88	1·16	0·99	1·05	1·07 ± 0·07	1·11
Brain	1·23	1·01	0·95	0·83	1·12	1·03 ± 0·04	1·00
Muscle	1·34	1·12	1·16	1·01	1·25	1·18 ± 0·06	0·90
Fat	0·26	0·25	0·23	0·23	0·23	0·24 ± 0·01	—
Mean†	1·43	1·02	1·16	1·02	1·12		1·09
± s.e.	0·06 ±	0·05 ±	0·07 ±	0·04 ±	0·04 ±		0·06 ±

* Value recorded corrected for 86 per cent recovery; all other tissues assumed to give 100 per cent recovery.

† Excluding fat.

‡ Only one animal, therefore no deviations can be calculated. The dose was 2·7 g. phenacetin; weight of dog not stated.

§ The final coloured solutions obtained from brain and fat were almost invariably opalescent. This was compensated by reading their optical density at 600 mμ and subtracting the value so obtained from the reading at 515 mμ. The reading of the diazo compound *per se* at 660 mμ is negligible, but the standards are routinely read the same way for this comparison.

Paracetamol is mainly excreted as the sulphate ether (about 67 per cent) and as the glucuronate (about 33 per cent), (Lester and Greenberg, 1947), and it seemed desirable to ensure that these metabolites would not interfere with the determination of non-conjugated drug. Addition, at

the level of 50 μg./ml., of either of these substances to whole blood, and to whole blood containing a standard amount of paracetamol was found not to increase the level of non-conjugated drug determined.

A number of samples were analysed by the methods of Brodie and Axelrod (1949) and also by the modification finally adopted. The results are shown in Table II. By analysing whole blood samples containing known amounts of drug over an extended period, the percentage standard deviation of the method, at the levels of 10, 20 and 40 μg./ml. whole blood was established as ±5–7 per cent.

TABLE II

COMPARISON BETWEEN METHODS OF ASSAY FOR NON-CONJUGATED PARACETAMOL

Subjects	Modified method μg./ml. whole blood	Brodie and Axelrod method μg./ml. whole blood
A	24·6	25·4
B	14·0	15·8
C	5·5	5·8
D	16·9	16·8
E	17·4	18·0
F	12·4	11·3
G	24·3	22·6

Reagents. Sodium sulphate (anhydrous granular), ether (Analar), 0·1N sodium hydroxide, 40 per cent sodium hydroxide, α-naphthol reagent, hydrochloric acid, *n*-butanol, isopentanol, and potassium chloride (Analar).

The ether and isopentanol were purified by shaking successively with 40 per cent sodium hydroxide, water, 10 per cent hydrochloric acid and water; they were finally dried with anhydrous sodium sulphate.

The α-naphthol reagent was prepared by reacting 1 ml. of 5 per cent ethanolic solution of α-naphthol with 10 mg. of potassium dichromate and 1 ml. of 2N hydrochloric acid for 3–5 min. 19 ml. of 5 per cent ethanolic solution of α-naphthol were then added.

Procedure. Anhydrous granular sodium sulphate was added slowly to a sample of whole blood (2 ml.) with mixing until a dry friable mass was obtained. After standing for 5 min. the mass was extracted in a Soxhlet thimble for 1 hr. with purified ether (100–150 ml.) containing 1·5 per cent of purified isopentanol. The extract was reduced to 50–70 ml. by evaporation on a steam-bath and then extracted with portions of aqueous 0·1N sodium hydroxide (5 ml., 2 ml.). The combined aqueous extracts were treated with concentrated hydrochloric acid (1·5 ml.) and heated in ½ in. diameter test tubes, covered with glass marbles, for 45 min. in a boiling water-bath. The solution was cooled to room temperature (25°), α-naphthol reagent (5 drops) and 40 per cent sodium hydroxide (2·5 ml.) were added and the mixture allowed to stand for 2–3 min. The solution was then saturated with solid potassium chloride and extracted with butanol (5 ml.). The extract was dried with anhydrous sodium sulphate to give a clear solution and the developed colour read on a spectrophotometer at 635 mμ. The paracetamol content of the original blood was obtained from a standard graph prepared by the addition of

known amounts of the drug to whole blood, and treatment by the foregoing method.

Relationship of Whole Blood to Plasma Levels in Man

In the original blood level studies reported by Brodie and Axelrod (1948, 1949), Lester and Greenberg (1947), Weikel (1958) and Carlo, Cambosos, Feeney and Smith (1955), whole blood or plasma levels were used, although none of these workers made any specific reference to the ratio between the two. It was therefore of interest to determine this ratio for man. Assays for paracetamol in blood and plasma from 9 subjects 45 min. after administration of 1 g. of the drug, are recorded in Table III, together with the ratios. This ratio remains virtually constant at about 1·1, whatever the amount of drug in the blood and plasma.

TABLE III

SINGLE ASSAY OF PARACETAMOL FROM BLOOD AND PLASMA 45 MIN. AFTER THE ADMINISTRATION OF 1 G. PARACETAMOL

Subject	Whole blood μg./ml.	Plasma μg./ml.	Ratio
1	4·5	4·5	1·0
2	26·6	25·5	1·04
3	12·8	13·5	0·95
4	17·3	15·8	1·09
5	18·0	17·3	1·04
6	25·2	21·5	1·17
7	20·2	18·3	1·11
8	24·8	22·1	1·11
9	23·2	22·1	1·05
		Mean	1·062

Discussion

In dogs, paracetamol is distributed evenly in all the tissues with the exception of fat. The mean values were higher than those reported by Brodie and Axelrod (1949); this may, however, be explained by the fact that these authors administered the drug as phenacetin and only part of this would have been converted to paracetamol at the time the dog was killed. Nor did they state the weight of the animal.

A more important finding was the confirmation that the mean tissue to plasma concentration ratio (again excluding fat) in the dog is slightly above unity throughout. In other words, the compound showed no special preference for any one tissue but occupied all of the available water space.

In man, we have demonstrated that the ratio between whole blood and plasma concentration is also close to 1·1. It follows that an estimate of tissue levels should be obtained by a simple determination of whole blood or plasma concentration—especially as Clark (1951) has shown that the drug is dealt with in similar fashion in both species.

The analytical method we now recommend for the examination of large numbers of samples has been shown to give results comparable to those of Lester and Greenberg (1947), and Brodie and Axelrod (1949), but with a spectrophotometric sensitivity about $2\frac{1}{2}$ times greater. Previous work has assumed that only non-conjugated paracetamol is

estimated. We have demonstrated that the two major conjugated forms of paracetamol do not interfere with the estimation of non-conjugated drug.

REFERENCES

Brodie, B. B. and Axelrod, J. (1948). *J. Pharmacol.*, **94**, 22–38.
Brodie, B. B. and Axelrod, J. (1949). *Ibid.*, **97**, 58–67.
Carlo, P. E., Cambosos, N. M., Feeney, G. C. and Smith, P. K. (1955). *J Amer. pharm. Ass., Sci. Ed.*, **44**, 396–399.
Clark, B. B. (1951). *Symposium on N-Acetyl-p-aminophenol*, p. 23–34. Institute for the Study of Analgesic and Sedative Drugs, Elkhart.
Lester, D. and Greenberg, L. A. (1947). *J. Pharmacol.*, **90**, 68–75.
Weikel, J. H. (1958). *J. Amer. pharm. Ass., Sci. Ed.*, **47**, 477–479.

THE ABSORPTION CHARACTERISTICS OF PARACETAMOL TABLETS IN MAN

By J. R. Gwilt,* A. Robertson,* L. Goldman† and A. W. Blanchard†

From the Analytical Department, Winthrop Laboratories, Newcastle upon Tyne 3 and the Medical Department,† The Winthrop Products Company, Surbiton, Surrey*

Received March 15, 1963

Paracetamol tablets from different sources showed widely differing absorption patterns in man. As judged by a blood concentration at 45 min. of less than 10 μg./ml. whole blood, low absorption was usually found in 25 per cent of subjects. This may be reduced to less than 10 per cent by a new paracetamol/sorbitol tablet. This combination produced higher average blood levels at 45 min. than crushed tablets of paracetamol. There appears to be a relation between the absorption of the drug and both age and weight; this variation can be reduced by paracetamol/sorbitol.

The work of Lester and Greenberg (1947) and Brodie and Axelrod (1948, 1949) established that paracetamol is the metabolite of acetanilide and phenacetin which is responsible for the analgesic and antipyretic effects of these compounds. Subsequently, paracetamol was studied by Clark (1951), Batterman and Grossman (1955), Carlo, Cambosos, Feeney and Smith (1955), Cornely and Ritter (1956), Orkin, Joseph and Helrich (1957) and Weikel (1958). Its absorption characteristics in man have now been further investigated.

Methods

Analytical Procedure

The analysis of non-conjugated paracetamol in whole blood was made using the method of Gwilt, Robertson and McChesney (1963).

Organisation of Panel

Because of the large number of venopunctures required, several panels of human volunteers were obtained from a large available population of both sexes between the ages of 20 and 50. There were 20 subjects in Panel 1, 52 in Panel 2, 144 in Panel 3 and 54 in Panel 4. A more detailed study of blood concentrations was made on Panel 4 and its composition is therefore given below.

Composition of Panel 4, 26 males, 28 Females

Age

20–25 yr.	25–30 yr.	30–35 yr.	35–40 yr.	40–45 yr.	45–50 yr.
7	17	7	7	7	9

Weight

7–9 st. (44–57 kg.)	9–11 st. (57–70 kg.)	11–13 st. (70–83 kg.)	13–15 st. (83–95 kg.)
8	26	14	6

Administration of Drug

Volunteers were instructed to take no medication in the 24-hr. period before the experiment. A random sampling showed that paracetamol was absent at the beginning of the experiment. A standard product was needed as a reference and therefore the most widely prescribed preparation of paracetamol was selected (Product P). Two 0·5 g. tablets were taken whole or powdered (No. 12 mesh) with 100 ml. of water. The drug was taken either on an empty stomach or 1–2 hr. after a breakfast of cereal, toast and a beverage. In comparative studies, tablets were given on successive days at the same time after the meal. Unless otherwise specified, all experiments were made after this standard meal.

At various intervals after taking the drug, 5 ml. samples of venous blood were withdrawn from the arm and immediately transferred to an oxalated container and shaken. The analysis was then made on a 2 ml. sample as soon as possible, the remainder of the blood being kept for duplicate assay where necessary.

Results

Using the 20 subjects in Panel 1, blood samples were withdrawn at 0·5, 1, 1·5 and 2·5 hr. after taking the drug. The average blood concentration at each of these times with their standard errors is shown in Fig. 1.

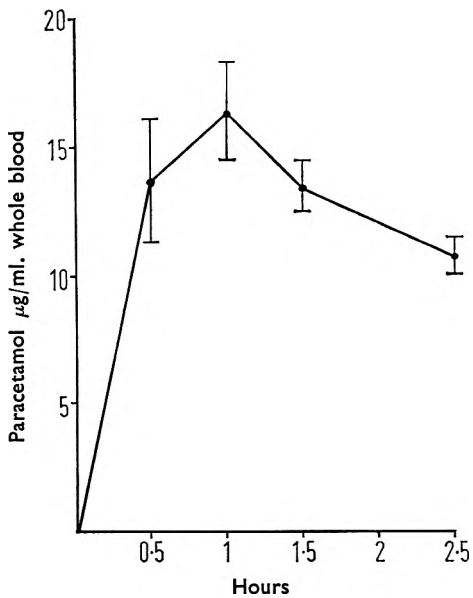


FIG 1. Average blood level (20 subjects) with ± s.e. Dosage 1000 mg. paracetamol as tablets (Product P).

The highest average concentration in the blood was reached between 30 and 90 min. As individual peak times varied, an arbitrary standard time of 45 min. was selected for future comparisons.

Absorption Distribution of Paracetamol

The standard dose of the drug (1 g.) was taken by the 144 subjects in Panel 3, blood samples being withdrawn at 45 min. after administration. The results are shown in Fig. 2 as a coarsely grouped histogram. The

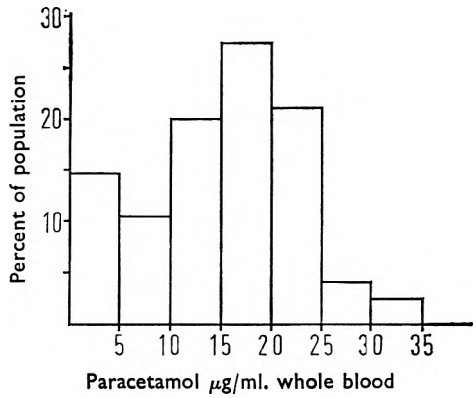


FIG. 2. Blood level distribution after administration of 1000 mg. paracetamol as Product P (144 subjects).

mean concentration was found to be 16 μg./ml. whole blood (s.e. 0·60). The results were further grouped into quartiles with approximate ranges of 0–10 μg./ml. (first quartile), 10–20 μg./ml. (second and third quartiles), over 20 μg./ml. (fourth quartile).

The second and third quartiles represent an approximate range of the mean of the whole distribution ± one standard deviation.

On this basis it was inferred that blood concentrations below 10 μg./ml. and above 20 μg./ml. whole blood probably represented poor and high absorption respectively. The low blood levels were not due to rapid excretion of the drug (unpublished observations).

Thirty-five of the panel of 144 subjects (24 per cent) would therefore appear to be low absorbers of paracetamol.

Blood Levels After Taking Other Paracetamol Tablets

Because of the high proportion (24 per cent) of subjects with a blood concentration of less than 10 μg./ml., seven other paracetamol products were studied. Samples of these, labelled K to R, were assayed for their physical characteristics. Weight, disintegration time and content of drug were within the limits recently laid down for the official B.P. standard.

Using Panel 2, each product, equivalent to 1 g. of drug, was given to each subject at a standard time, a single blood sample being withdrawn at 45 min. The means and standard errors are shown in Table I. The results indicate that most of the paracetamol products produced a mean blood concentration at 45 min. of 15–16 μg./ml. 95 per cent confidence limits for the means of the 8 products tested are 2·02 μg./ml. and there is obviously no significant difference between the means of Products L, M,

TABLE I

MEAN BLOOD LEVELS, μG./ML. WHOLE BLOOD, 45 MIN. AFTER ADMINISTRATION OF EIGHT PARACETAMOL PREPARATIONS IN A DOSE OF 1 G. EACH RESULT IS AN AVERAGE OF 45 SUBJECTS

Product	Mean, μg./ml.	Standard error, μg./ml.
K	12·6	0·75
L	15·0	0·94
M	15·0	0·78
N	15·4	0·87
O	16·2	0·93
P*	16·0	0·60
Q	18·0	0·82
R	19·3	0·83
Grand Mean ..	15·9	0·82

* Average of 144 subjects.

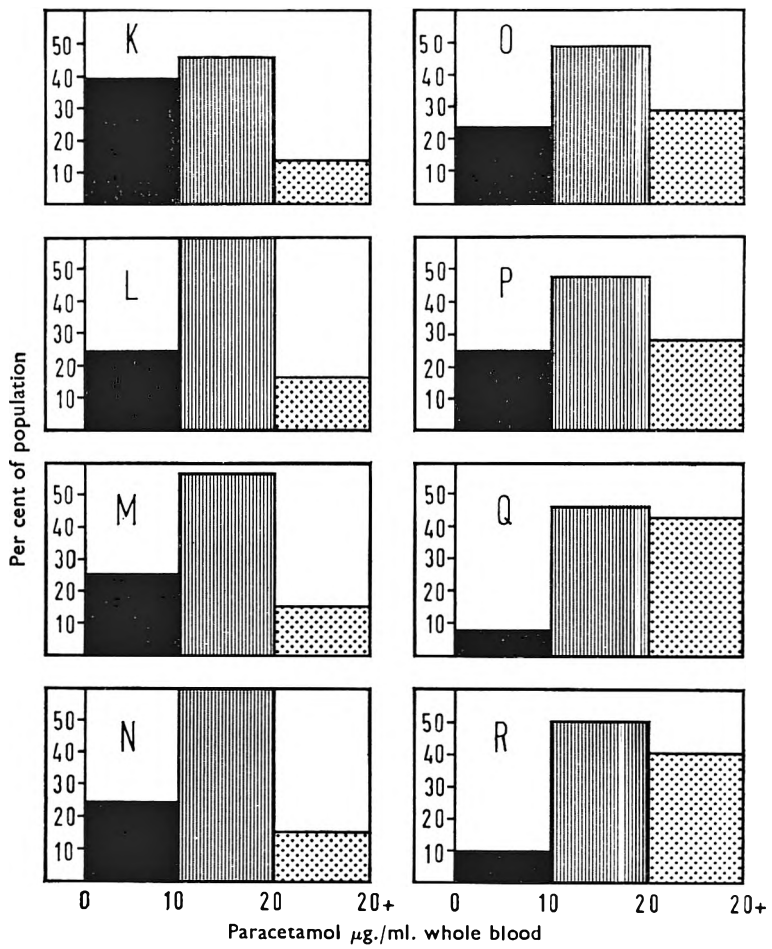


FIG. 3. Blood level distribution for eight currently available paracetamol tablets (dose 1000 mg.).

N, O and P. The mean for product K, is significantly smaller than the rest ($P<0.05$), while that for R is significantly greater than the rest ($P<0.05$); Product Q also approaches a significant difference at the 95 per cent level.

In Fig. 3, the results are shown as coarsely grouped histograms. As judged by a 45 min. blood concentration of less than 10 μg./ml. whole blood, the range of poor absorption varies from 8–39 per cent of all subjects tested. With most of the products (L, M, N, O and P), poor absorption occurs in approximately 25 per cent of subjects. Because of the marked variation in absorption of these products, a compound was sought which would reduce the percentage of poor or low absorbers.

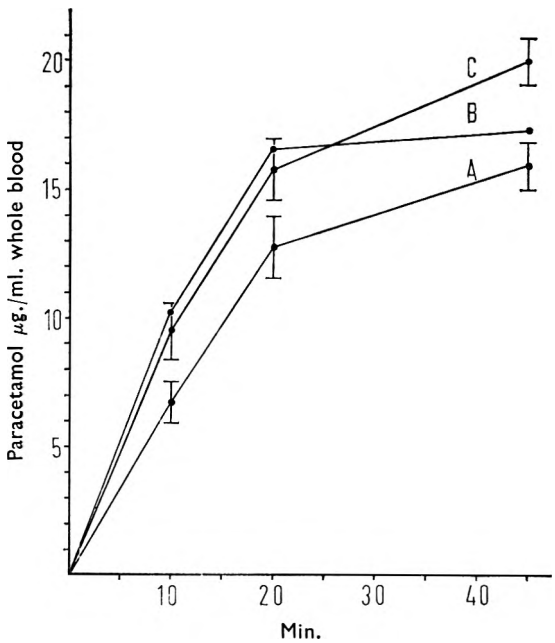


FIG. 4. Average blood levels (54 subjects) with ± s.e. for paracetamol and paracetamol/sorbitol whole tablets, 10, 20 and 45 min. after administration (dose 1000 mg.).

A, Paracetamol tablets whole.
B, Paracetamol tablets crushed.
C, Paracetamol/sorbitol tablets whole.

Studies on a New Paracetamol Tablet

It has been previously reported (Boger, Brashear and Gavin, 1959, and Herbert, Bierfass, Wasserman, Estren and Brody, 1959), that the absorption of some drugs can be increased by the hexahydric alcohol, sorbitol. Experiments were therefore made using varying amounts of sorbitol and paracetamol and, eventually, a tablet containing 500 mg. paracetamol and 100 mg. sorbitol was submitted to comparative blood studies. This new tablet had an acceptable disintegration time (B.P. method).

As variations in the results from whole tablet studies may be due purely to disintegration phenomena, a comparison of whole tablets with crushed tablets was also made. The 54 subjects in Panel 4 were used, the tablets being taken on an empty stomach. Blood samples were withdrawn at 10, 20 and 45 min. Three preparations were compared, viz., the new paracetamol/sorbitol tablet, whole, and paracetamol (Product P) whole and crushed. The order of administration of the three preparations was randomised to eliminate order effect.

The average blood concentrations for these three preparations are shown in Fig. 4, each point on the curve being an average of 54 readings, together with the standard errors for paracetamol (whole) and paracetamol/sorbitol. The results were submitted to analysis of variance to test their significance and the calculations are given in Table II. The analysis of

TABLE II

ANALYSIS OF VARIANCE BETWEEN PARACETAMOL TABLETS WHOLE, PARACETAMOL TABLETS CRUSHED AND PARACETAMOL/SORBITOL TABLETS

Source	Sum of squares	d.f.	Mean square	Variance ratio
Between subjects	11,791·94	53	(222·49)	
Between times	6,854·41	2	(3,427·20)	
Between products	1,111·85	2	(555·93)	
TxS Interaction	3,331·79	106	31·43	1·69*
TxP Interaction	291·38	4	72·85	3·91*
SxP Interaction	5,018·81	106	47·35	2·54*
TxSxP	3,949·17	212	18·63	
Total	32,349·39	485		

* Significant at $P<0.001$.

variance of the results from 10, 20 and 45 min. samples showed that both the combination and crushed drug alone were significantly better absorbed ($P < 0.05$) than whole tablets of the drug alone at 10 and 20 min. At 45 min., however, paracetamol/sorbitol was significantly better absorbed ($P < 0.05$) than paracetamol, either crushed or whole.

As these were studies on an empty stomach, the combination was then given to the 52 subjects in Panel 2, so that a true comparison could be obtained with the results from the other products tested. Tablets were given at the same time interval after a morning meal as in other studies. The results shown in Fig. 5 in histogram form may be compared with Fig. 3.

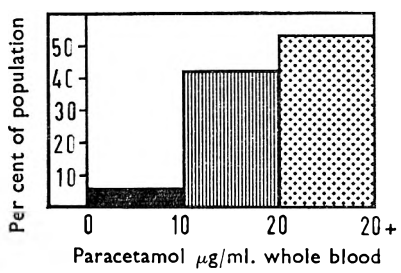


FIG. 5. Blood level distribution 45 min. after administration of paracetamol/sorbitol tablets (dose 1000 mg.). Compare Fig. 3.

With paracetamol/sorbitol the number of low or poor absorbers was reduced to 6 per cent. The average blood concentration at 45 min. was 20·8 μg./ml. whole blood (s.e. 0·84), which is significantly higher ($P < 0.05$) than the grand mean recorded for the 8 products previously tested (Table I).

Effect of Other Variables

Weight. The results from Panel 4 (54 subjects) for the drug as whole and crushed tablets and with sorbitol as whole tablets (Fig. 4) were examined for the effect of weight. For all presentations, blood concentration was found to be lower in the six subjects weighing 83–95 kg. and higher in the eight subjects weighing 44–57 kg. The difference was further investigated by calculating regression coefficients. Each of these was found to be highly significant ($P < 0.001$). They are expressed graphically in Fig. 6. There is obviously no difference between crushed

FIG. 6. Combined graph of trend lines for regression of blood level on body weight. (Dose 1000 mg.).

A, Paracetamol tablets whole.
B, Paracetamol tablets crushed.
C, Paracetamol/sorbitol tablets whole.

tablets of paracetamol and whole tablets of paracetamol/sorbitol. The difference between paracetamol whole tablets and the combination, however, approaches significance at a 95 per cent level. It may be therefore that the dependence of blood concentration on weight can be improved with crushed tablets or with the combination.

Age. Using the same data, an assessment was made of the effect of age. The results are expressed graphically in Fig. 7.

Both for whole and crushed tablets, absorption appeared poorest in the younger and older age groups and best in age groups 30–40; whereas with paracetamol/sorbitol there was an approximately linear relationship between age and blood concentration.

Dose size. To test whether a similar reduction in poor absorption could have been achieved more simply by increasing the amount of paracetamol, 1,500 mg. (as Product P) was given to 50 of the subjects in Panel 4. The tablets were taken on an empty stomach and the results compared with those previously obtained for 1,000 mg. of drug alone and with sorbitol.

As expected, increasing the dose to 1,500 mg. produced a higher average blood concentration (23·8 μg./ml. whole blood, s.e. 1·40), than either 1,000 mg. of drug or drug plus sorbitol. However, the increased dose did not reduce below 10 per cent the number of those subjects with a blood concentration of less than 10 μg./ml. whole blood.

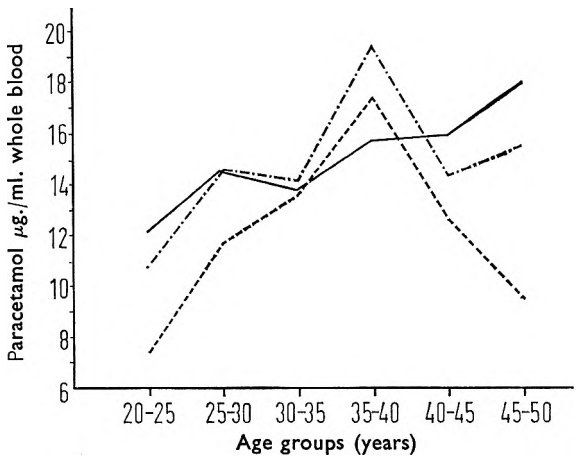


FIG. 7. Average of 10, 20 and 45 min. readings related to age groups.
——————— Paracetamol/sorbitol tablets.
·—·—·—·—· Paracetamol tablets crushed.
— — — — Paracetamol tablets whole.

Stomach contents. The results on absorption from an empty stomach and after a normal meal were analysed to determine the effect of stomach contents on absorption. For whole tablets of product P the results were 15·9 and 16·0 and for the combination 20·1 and 20·8 μg./ml. respectively. There is no significant difference for paracetamol or paracetamol/sorbitol whether taken on an empty stomach or after a meal. However, the differences between drug alone and in combination under both conditions are highly significant ($P < 0.001$).

DISCUSSION

We have shown previously (Gwilt and others, 1963) that the tissue levels of paracetamol may be inferred from blood level estimations. As judged by a 45 min. blood concentration of less than 10 μg./ml., the average figure for poor absorption was about 25 per cent with various paracetamol preparations. A new paracetamol/sorbitol tablet produced higher blood concentrations than the drug alone and thus raised the blood concentrations of subjects who previously had low levels to a concentration comparable to that of subjects who absorbed the drug well. Similarly, the average blood concentration at 45 min. was found to be 20·8 μg./ml., a significantly higher level than had been obtained with ordinary tablets tested in comparable dosage.

Indeed, at 45 min., paracetamol/sorbitol was significantly better absorbed than the drug alone as crushed or whole tablets. It is interesting to speculate on these findings. If the addition of sorbitol acted merely as a dispersal agent, then the absorption patterns of the new tablet should resemble that of a crushed tablet. From our studies, it would appear that sorbitol, in addition to dispersal, acts in some way on the metabolism of the drug.

It could be maintained that the same advantage might be achieved more simply by the administration of a larger dose of paracetamol but we have shown that even in a dose of 1,500 mg., it does not reduce the percentage of low absorption more than can be achieved with the combination for a lesser dose of the drug.

Comparing the three types of medication, paracetamol whole and crushed and paracetamol/sorbitol, with respect to age and weight, there was an inversely linear effect on blood concentration with increasing weight, i.e., the heavier the subject the lower the blood concentration. Although this is what would normally be expected, this dependence could be reduced either by crushing the tablet or by using the combination. There also appeared to be a dependence on age, the best absorption with the drug alone taking place in those subjects between the ages of 30–40. With the combination, however, there was an approximately linear relation between age and blood concentration, a finding to be expected for both preparations because the metabolic rate is likely to be higher in the younger than in the older subject. There is no obvious explanation for the poor results in the younger and older subject with paracetamol.

References

Batterman, R. C. and Grossman, A. J. (1955). *Fed. Proc.*, **14**, 316.

Boger, W. P., Brashear, D. S. and Gavin, J. J. (1959). *Amer. J. Clin. Nutrit.*, **7**, 318–324.

Brodie, B. B. and Axelrod, J. (1948). *J. Pharmacol.*, **94**, 22–38.

Brodie, B. B. and Axelrod, J. (1949). *Ibid.*, **97**, 58–67.

Carlo, P. E., Cambosos, N. M., Feeney, G. C. and Smith, P. K. (1955). *J. Amer. pharm. Ass., Sci. Ed.*, **44**, 396–399.

Clark, B. B. (1951). *Symposium on* N-*acetyl*-p-*aminophenol*, 23–34. Institute for the Study of Analgesic and Sedative Drugs, Elkhart.

Cornely, D. A. and Ritter, J. A. (1956). *J. Amer. med. Ass.*, **160**, 1219–1221.

Gwilt, J. R., Robertson, A. and McChesney, E. W. (1963). *J. Pharm. Pharmacol.*, **15**, 440–444.

Herbert, V., Bierfass, M., Wasserman, L. R., Estren, S. and Brody, E. (1959). *Amer. J. clin. Nutrit.*, **7**, 325–327.

Lester, D. and Greenberg, L. A. (1947). *J. Pharmacol.*, **90**, 68–75.

Orkin, L. R., Joseph, S. I. and Helrich, M. (1957). *New York State J. Med.*, 71–73.

Weikel, J. H. (1958). *J. Amer. pharm. Ass., Sci. Ed.*, **47**, 477–479.

EFFECT UPON THE ANALGESIC ACTION OF RESERPINE OF CENTRAL NERVOUS SYSTEM STIMULANTS AND DRUGS AFFECTING THE METABOLISM OF CATECHOL- AND INDOLE-AMINES

BY J. GARCIA LEME AND M. ROCHA E SILVA

From the Department of Pharmacology, Faculty of Medicine, U.S.P. Ribeirão Prêto, E.S. Paulo, Brasil

Received December 27, 1962

The effect upon the analgesic action of reserpine of central nervous system stimulants and of drugs affecting the metabolism of catechol- and indole-amines as measured in mice by a hot-plate method has been analysed. The analgesic effect, which has a maximal intensity 48 hr. after injection of the alkaloid, is partially or totally counteracted by MAO inhibitors, LSD-25 and 5-hydroxytryptophan. Central nervous system stimulants given to reserpinised mice before each test were effective in reducing the reaction time to the heat stimulation only 24 and 48 hr. after reserpine. DOPA showed no significant ability to reduce the analgesic effect of reserpine. In the light of these findings a suggestion has been made that 5-hydroxytryptamine rather than catecholamines would be involved in the mechanism of the analgesic effect due to reserpine and since central nervous system stimulants are able to reduce this action initially but are ineffective later, part of the effect is thought to be due to sedation of the animals, the participation of the stimulants being confined to a direct antagonism against the sedation. The lasting analgesic effect is supposed to correspond to a lack of 5-hydroxytryptamine in the cerebral structures.

IN a previous publication (Leme and Rocha e Silva, 1961) we described experiments in mice which showed increased reaction time (RT), under the influence of reserpine, to exposure to a hot-plate at 55°. This prolongation of the RT developed slowly, being maximal 48 hr. after the injection of reserpine, and returning slowly to normal levels 5–6 days after treatment. That this effect was due to a central analgesic effect was supported by the observation that reserpine potentiates morphine 2 hr. after their injection, while 24 to 48 hr. after reserpine its effects sum with those of morphine. The analgesic effect of reserpine differs from that of morphine, in being of slow onset and persistent for days, while that of morphine develops quickly and disappears after 1–2 hr. Since reserpine is known to deplete tissues of their catechol- and indole-amines (Pletscher, Shore and Brodie, 1956; Paasonen and Vogt, 1956), it seemed logical to assume that its analgesic effect might be related to its amine-releasing activity in the central nervous system. An interesting coincidence is the similar times needed for the replenishment of catechol- and indole-amines in the central nervous system after depletion by reserpine, observed in rabbits by Pletscher, Shore and Brodie (1956), and the return to normal of the RT in mice submitted to thermal stimulus.

We have now attempted to amplify our knowledge of the possible mechanism of the analgesic action of reserpine by analysing the action of compounds which might interfere with the metabolism of 5-hydroxytryptamine (5-HT) and catecholamines, such as inhibitors of monoamine

oxidase (MAO), 5-hydroxytryptophan (5-HTP), 3,4-dihydroxyphenylalanine (DOPA), LSD–25 and other central nervous system stimulants, such as sympathomimetic amines or bemegride.

Materials and Methods

Male white mice, 15 to 20 g., were tested by the hot-plate (55°) method (Leme and Rocha e Silva, 1961). The RT was measured from the moment the animal was placed on the plate until it presented a sign of discomfort characterised by a licking of both front paws simultaneously or a sudden jump.

Solutions in distilled water were prepared in concentrations such that each animal received a maximum of 1 ml. injected intraperitoneally.

Reserpine was given in a single dose of 5 mg./kg. of the drug. The RT was measured 24, 48 and 72 hr. after the injection of reserpine in all instances.

Animals treated with MAO inhibitors and reserpine received two divided doses of the inhibitors 24 and 2 hr. before reserpine and the RT was measured as described.

Animals, previously injected with reserpine, received central nervous system stimulants in three doses, each given 30 min. before the tests for RT.

Mice treated with reserpine and LSD–25 were distributed into two groups: one received reserpine and a single dose of LSD–25 30 min. before the first exposure to the hot-plate; the other received reserpine and then a dose of LSD–25 was given 30 min. before each of the three RT tests.

Animals treated with 5-HTP or DOPA received a dose a day for 3 days before the reserpine. The RT was then measured as described.

A control group (no treatment) of 100 animals was exposed to the hot-plate three times at intervals of 24 hr. The RT had a mean $\pm$ s.e. of $10{\cdot}0 \pm 0{\cdot}4$ sec. in the first exposure, $11{\cdot}9 \pm 0{\cdot}5$ sec. in the second and $12{\cdot}3 \pm 0{\cdot}6$ in the third.

The following substances were used: reserpine, iproniazid, DL-trans-2-phenylcyclopropylamine (SKF385), pheniprazine, *N*-2-methyl-1,4-benzodioxane-*N*-benzylhydrazine tartrate (2596 IS), methylamphetamine hydrochloride, ephedrine sulphate, bemegride, D-lysergic acid diethylamide tartrate (LSD–25), DL-5-hydroxytryptophan, DL-3,4-dihydroxyphenylalanine.

Results

Effect of reserpine on RT *at* 55°. We confirmed the previous results (Leme and Rocha e Silva, 1961) showing that reserpine in a single injection prolongs the RT for several days.

Ninety animals treated with reserpine showed an increase in RT (Table I) when compared with a control group (100 animals). The maximum increase is around 48 hr. after the injection of the drug, returning to normal after a few days. The observations were limited to 72 hr. after the injection.

Effect of MAO *inhibitors on* RT *at* 55° *of reserpine-treated mice.* All four MAO inhibitors, given before reserpine, counteracted partially or totally

TABLE I

REACTION TIMES OF A GROUP OF ANIMALS INJECTED WITH 5 MG./KG. RESERPINE, COMPARED WITH A CONTROL GROUP

Treatment	Reaction time (sec.)			Number of animals
	24 hr. after reserpine (mean ± s.e.)	48 hr. after reserpine (mean ± s.e.)	72 hr. after reserpine (mean ± s.e.)	
Reserpine: 5 mg./kg. (single dose) ..	16·9 ± 1·0	26·7 ± 1·3	18·2 ± 0·8	90
Controls (no treatment)	1st exposure 10·0 ± 0·4	2nd exposure 11·9 ± 0·5	3rd exposure 12·3 ± 0·6	100

the prolongation of RT produced by reserpine. Table II summarises the results.

Forty-five animals were given iproniazid, 200 mg./kg., in two equal doses 24 and 2 hr. before reserpine showed a significant reduction of RT.

Fifty animals received SKF385, 4 mg./kg., in two equal doses 24 and 2 hr. before reserpine showed a decrease in the RT mean values.

TABLE II

REACTION TIMES OF ANIMALS SUBMITTED TO COMBINED TREATMENT: MAO INHIBITORS + RESERPINE

Treatment before 5 mg./kg. reserpine	Reaction time (sec.)			Number of animals
	24 hr. after reserpine (mean ± s.e.)	48 hr. after reserpine (mean ± s.e.)	72 hr. after reserpine (mean ± s.e.)	
Iproniazid, total dose: 200 mg./kg. ..	12·5 ± 0·7	17·9 ± 0·9	14·8 ± 0·7	45
SKF 385, total dose: 4 mg./kg. ..	10·6 ± 0·5	14·6 ± 0·8	12·6 ± 0·6	50
Pheniprazine, total dose: 4 mg./kg. ..	9·7 ± 0·4	11·1 ± 0·5	9·6 ± 0·5	50
2596 IS, total dose: 20 mg./kg. ..	9·4 ± 0·4	10·7 ± 0·4	10·7 ± 0·5	50

Fifty animals were injected with two doses of pheniprazine, 2 mg./kg., 24 and 2 hr. before reserpine. This drug proved to be more potent than SKF385 (weight/weight) in reducing the RT mean values.

Two doses of 2596IS, 10 mg./kg., 24 and 2 hr. before reserpine in 50 animals were effective in reducing the RT mean values.

TABLE III

REACTION TIMES OF ANIMALS SUBMITTED TO COMBINED TREATMENT: RESERPINE + CNS STIMULANTS

Treatment	Reaction time (sec.)			Number of animals
	24 hr. after reserpine (mean ± s.e.)	48 hr. after reserpine (mean ± s.e.)	72 hr. after reserpine (mean ± s.e.)	
Reserpine: 5 mg./kg. + methyl-amphetamine: 3 doses (10 mg./kg./24 hr.)	15·1 ± 1·1	20·3 ± 1·2	16·7 ± 1·1	50
Reserpine: 5 mg./kg. + ephedrine: 3 doses (10 mg./kg./24 hr.)	12·0 ± 1·4	16·5 ± 1·0	16·3 ± 1·0	50
Reserpine: 5 mg./kg. + bemegride: 3 doses (1 mg./kg./24 hr.)	9·4 ± 0·7	16·6 ± 1·4	17·3 ± 1·6	50

Effect of central nervous system stimulants on RT *at 55° of reserpine-treated mice.* Methylamphetamine and ephedrine, 10 mg./kg., and bemegride, 1 mg./kg., injected 30 min. before the tests on the hot-plate in three groups of 50 animals previously treated with reserpine, caused a decrease in the mean values of RT, methylamphetamine being the least potent (Table III).

Effect of LSD–25 on RT *at 55° of reserpine-treated mice.* After reserpine, a single dose of 500 μg./kg. LSD–25, 30 min. before the first exposure to the plate, or three daily doses of 500 μg./kg. 30 min. before the RT tests, were effective in reducing the mean values of the RT. No significant difference was seen between the two groups (50 animals in each group) of mice. Thus, a single dose injected at the onset of the assay was as potent as three doses of the drug, given at 24 hr. intervals, decreasing the RT values of reserpine-treated mice, as seen in Table IV.

TABLE IV

REACTION TIMES OF MICE SUBMITTED TO COMBINED TREATMENT: RESERPINE + LSD–25

Treatment	Reaction time (sec.) 24 hr. after reserpine (mean ± s.e.)	48 hr. after reserpine (mean ± s.e.)	72 hr. after reserpine (mean ± s.e.)	Number of animals
Reserpine: 5 mg./kg. + LSD: one dose (500 μg./kg.)	12·6 ± 0·8*	16·0 ± 1·2	15·3 ± 1·0	50
Reserpine: 5 mg./kg. + LSD: 3 doses (500 μg./kg./24 hr.)	11·7 ± 0·8*	15·3 ± 1·0†	13·7 ± 0·9‡	50

* 45 min. after 1st dose LSD. † 45 min. after 2nd dose LSD. ‡ 45 min. after 3rd dose LSD.

Effect of 5-HTP *and* DOPA *on* RT *at 55° of reserpine-treated mice.* Three doses of 5-HTP, 100 mg./kg./24 hr., to 95 animals, before reserpine, reduced the RT values (Table V), while DOPA similarly injected into 75 animals showed no ability to reduce the RT prolonged by reserpine (Table V).

TABLE V

REACTION TIMES OF ANIMALS SUBMITTED TO COMBINED TREATMENT: 5-HTP OR DOPA AND RESERPINE

Treatment	Reaction time (sec.) 24 hr. after reserpine (mean ± s.e.)	48 hr. after reserpine (mean ± s.e.)	72 hr. after reserpine (mean ± s.e.)	Number of animals
5-HTP: 3 doses (100 mg./kg./24 hr.) + reserpine: 5 mg./kg.	13·5 ± 1·0	16·6 ± 1·0	14·3 ± 0·9	95
DOPA: 3 doses (100 mg./kg./24 hr.) + reserpine: 5 mg./kg.	20·9 ± 1·6	20·9 ± 1·3	20·2 ± 1·3	75

CONCLUSIONS AND DISCUSSION

Our results show that the analgesic action of reserpine, as measured in mice by a prolongation of RT when the animals are exposed to a hot-plate at 55°, is influenced by drugs given in association with the alkaloid.

The MAO inhibitors, iproniazid, SKF385, pheniprazine and 2596IS, decreased the RT values. No attempt has been made to relate the potency of these drugs to the analgesic action of reserpine.

The central nervous system stimulants were also able to reduce the analgesic effect. This reduction was observed 24 and 48 hr. after reserpine, but no significant reduction was seen 72 hr. after the alkaloid. Since methylamphetamine and ephedrine are also inhibitors of MAO (Gaddum and Kwiatkowski, 1938; Blaschko, 1952), at least part of their action could be due to this effect. However, bemegride which is devoid of such an action has been shown to be more potent in reducing RT after reserpine than the sympathomimetic amines.

LSD–25 displays a potent inhibitory effect upon the analgesic action of reserpine. No difference was observed when used as a single dose or with repeated daily doses.

5-HTP greatly inhibited the analgesic effect of reserpine, while DOPA had no action.

The analgesic action of reserpine as measured by the prolongation of the RT when mice are exposed to the hot-plate, shows peculiarities that might be discussed in the light of the above findings. The maximum effect is attained 48 hr. after the injection of the drug, and therefore might be interpreted as an indirect consequence of the action of the drug upon the physiological status of the reacting structures, either locally or in the central pathways for the painful stimuli. Since reserpine is known to deplete tissues of their stores of catechol- and indole-amines, the prolongation of the RT to heat stimulation, might be due to such a depletion in the central nervous system. In that case, the changes in RT observed after reserpine might reflect the degree of depletion of those amines in the brain. Interference by drugs affecting the metabolism of catechol- and indole-amines might give information about such a mechanism.

We have demonstrated this analgesic effect to be inhibited by MAO inhibitors. Though catechol-*O*-methyl transferase is concerned with the first step in a major metabolic pathway of adrenaline and noradrenaline (Axelrod, 1957; Axelrod and Tomchick, 1958), the inhibition of MAO has been demonstrated to effect a rise in the levels of these amines in tissues (Shore, Mead, Kuntzman, Spector and Brodie, 1957; Leroy and Schaepdryver, 1961). In a parallel way, the 5-HT content of brain tissues is increased when MAO inhibitors are administered to animals (Pletscher, Göschke, Gey and Thölen, 1961; Spector, Shore and Brodie, 1960). If these drugs are given before reserpine, there is less depletion of catechol- and indole-amines (Weil-Malherbe, Posner and Bowles, 1961; Brodie, Pletscher and Shore, 1956). That MAO inhibitors are effective in reducing the analgesic effect of reserpine could be taken as an indirect suggestion of the role played by catecholamines and 5-HT in this analgesic action.

5-HTP besides being a precursor of 5-HT, easily penetrates the blood-brain barrier. This drug was found to strongly reduce the analgesic action of reserpine. This could be additional evidence of the role of 5-HT in this action.

As far as the participation of catecholamines is concerned, DOPA, the

precursor of these amines, which behaves like 5-HTP in relation to the blood-brain barrier, did not reduce significantly the RT of reserpine-treated mice. Rather, it potentiated the effect of reserpine in the first 24 hr. This could lead us to doubt a relation between a lack of catecholamines and the analgesic effect of reserpine, or at least to exclude a primary role for them in this phenomenon.

LSD–25 has been demonstrated by Freedman (1961) and Freedman and Giarman (1962) to induce a rise in levels of rat brain 5-HT and to stimulate repletion of 5-HT after reserpine release. If we assume that depletion of 5-HT could play a role in the production of the analgesic effect, we could easily understand the action of LSD–25 in our experiments. Nevertheless, we have to remember that LSD–25 is a potent central stimulant and that we have seen central stimulants to be effective in reducing the analgesic action of reserpine.

The facts mentioned above suggest that 5-HT more than catecholamines would be involved in the mechanism of the analgesic action of reserpine. Depletion of 5-HT after reserpine injection seems to be closely related to this action. This fact does not allow us, however, to postulate that decreased levels of 5-HT in the central nervous system are the cause of analgesia.

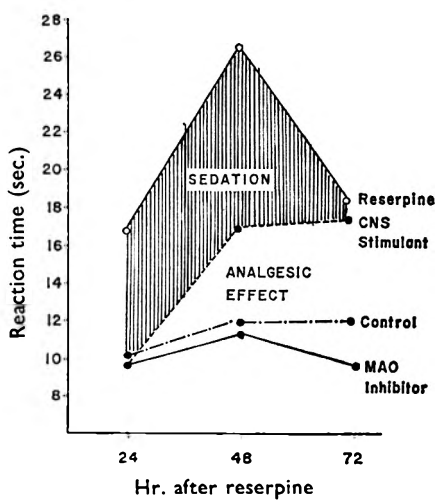


FIG. 1. Theoretical interpretation of the effects of the CNS stimulants and MAO inhibitors upon the RT of animals submitted to a single injection of reserpine. The data are taken from Tables I and II.

Since central nervous system stimulants are able to reduce the RT increased by reserpine, one might assume that part of the effect observed is due to sedation of the animals clearly seen in the first period after the administration of reserpine. As these drugs had much less or no effect at the second and third periods, when reserpine has already been partially or totally eliminated, it might be thought that the effect of the central

nervous system stimulants is confined to a direct antagonism of the sedation produced by reserpine, and therefore has little to do with the more lasting analgesic effect possibly due to a lack of 5-HT in the cerebral structures. A pictorial interpretation of the phenomenon is presented in Fig. 1, in which the shaded zone corresponds to the sedation effect which might be completely counteracted by the central nervous system stimulants (bemegride and ephedrine). Along these lines, the antagonising effect of the inhibitors of MAO (pheniprazine and 2596IS) would extend over both phases of the phenomenon, as it is known that they are also stimulants of the central nervous system. However, with the experimental set up we have used we could not measure the exact contributions of the two phases of the phenomenon to the observed extension of the RT, and the scheme of Fig. 1, only sets the limits beyond which a simple sedation would not be enough to explain the delayed analgesic effect induced by reserpine.

REFERENCES

Axelrod, J. (1957). *Science*, **126**, 400–401.
Axelrod, J. and Tomchick, R. (1958). *J. biol. Chem.*, **233**, 702–705.
Blaschko, H. (1952). *Pharmacol. Rev.*, **4**, 415–458.
Brodie, B. B., Pletscher, A. and Shore, P. A. (1956). *J. Pharmacol.*, **116**, 9.
Freedman, D. X. (1961). *Ibid.*, **134**, 160–166.
Freedman, D. X. and Giarman, N. J. (1962). *Ann. N.Y. Acad. Sci.*, **96**, 98–107.
Gaddum, J. H. and Kwiatkowski, H. (1938). *J. Physiol.*, **94**, 87–100.
Leme, J. G. and Rocha e Silva, M. (1961). *J. Pharm. Pharmacol.*, **13**, 734–742.
Leroy, J. G. and Schaepdryver, A. F. (1961). *Arch. Int. Pharmacodyn.*, **130**, 231–234.
Paasonen, M. K. and Vogt, M. (1956). *J. Physiol.*, **131**, 617–626.
Pletscher, A., Göschke, H., Gey, K. F. and Thölen, H. (1961). *Med. Exp.*, **4**, 113–117.
Pletscher, A., Shore, P. A. and Brodie, B. B. (1956). *J. Pharmacol.*, **116**, 84–89.
Shore, P. A., Mead, J. A. R., Kuntzman, R. G., Spector, S. and Brodie, B. B. (1957). *Science*, **126**, 1063–1064.
Spector, S., Shore, P. A. and Brodie, B. B. (1960). *J. Pharmacol.*, **127**, 15–21.
Weil-Malherbe, H., Posner, H. S. and Bowles, G. R. (1961). *Ibid.*, **132**, 278–286.

LOSS OF BIOLOGICAL ACTIVITY OF APOMORPHINE FROM AUTO-OXIDATION

By A. M. Burkman

From the Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois, Chicago 12, Illinois, U.S.A.

Received January 11, 1963

Buffered and unbuffered solutions of apomorphine hydrochloride in physiological saline were incubated at 30° for 61 days and samples were periodically withdrawn for both chemical and biological assay. Within the limits of error, the loss of biological activity, as measured by the gradual diminution in the intensity of the pecking syndrome in pigeons, paralleled the disappearance of unoxidised apomorphine. The un-buffered solutions retained approximately 75 per cent of their biological activity after 60 days incubation while solutions buffered initially to pH 6·0 were biologically inert after 16 days. Extracted and concentrated degradation product(s) when administered to pigeons failed to initiate the pecking syndrome.

The development of a readily quantifiable measure of pecking syndrome intensity in pigeons provides us with a useful and reliable index of apomorphine potency. From an experimental point of view, it has proven to be more reliable than those methods utilising the familiar emetic response in dogs. Using the pigeon assay we have been able to effectively monitor apomorphine activity under a variety of experimental conditions and have adapted the method as a screening procedure for potential anti-emetic, psycholeptic agents (Burkman, 1961a, 1962). The applicability of such a method is based upon an apparent parallelism existing between the avian response and emesis in mammals.

The recent investigations of apomorphine by Kaul, Brochmann-Hanssen and Way (1961a,b,c), have further stimulated interest in the activity of apomorphine metabolites as pecking syndrome stimulants. The present communication describes a study undertaken to determine whether the products of spontaneous oxidation retain the ability to initiate the avian syndrome.

Experimental Methods

Solutions. Unbuffered solutions of 100 ml. quantities of 0·9 per cent sodium chloride contained 50 mg. of apomorphine hydrochloride. Buffered solutions were prepared by dissolving 50 mg. of apomorphine hydrochloride in 50 ml. of 0·9 per cent sodium chloride, adding 4 ml. of buffer, pH 6·0 (McIlvaine, 1921) and sufficient saline to make 100 ml. Four preparations of both buffered and unbuffered solutions served as experimental replicates.

Incubation. Solutions were placed in 250 ml. glass stoppered Erlenmeyer flasks immersed to the neck in a water bath maintained at 30 ± 0·1°. The flasks were charged daily with oxygen and subjected to intermittent agitation to discourage adhesion of degradation products to the inner walls.

Determination of unoxidised apomorphine. The spectrophotometric method of Kaul, Brochmann-Hanssen and Way (1959) was used. The only changes made were in the volumes of extraction solvents and buffer used to better accommodate the quantities of apomorphine being assayed. Samples from freshly prepared solutions, assayed at zero incubation time, served as controls. The results from subsequent determinations were expressed as per cent of their control. Thus, the disappearance of apomorphine served as the measure of chemical degradation.

Biological assay. Groups of adult domestic pigeons (*Columba livia*) of mixed sex were initially screened for apomorphine sensitivity at the 0·5 mg./kg. level and resistant animals discarded. Four birds served as assay subjects for *each* of the incubated preparations of apomorphine (a total of 32 pigeons). Samples of solutions were periodically removed and administered to birds in doses of 0·5 mg./kg., assuming a constant concentration of 0·5 mg. of apomorphine hydrochloride per ml. throughout the incubation period. The cumulative pecking responses evoked by the freshly prepared solutions (zero incubation time) represented control values against which subsequent measurements were compared. The results were expressed as per cent of control.

Quantitative assay of the pecking syndrome has been described elsewhere (Burkman, 1961b). All injections were made into the peritoneal cavity.

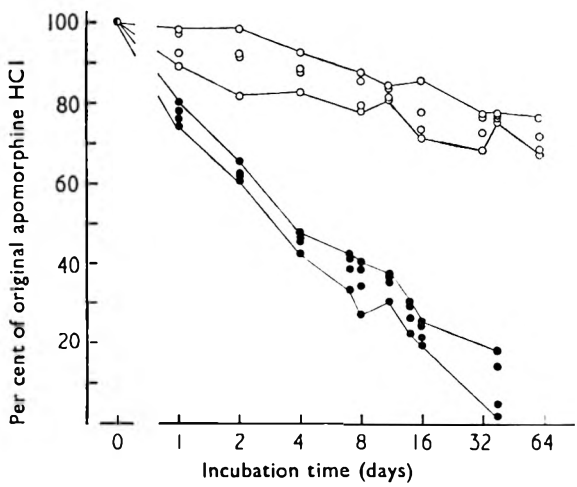


FIG. 1. Apomorphine HCl degradation at 30°. Original concentration: 0·5 mg./ml. of 0·9 per cent sodium chloride solution. —○— Unbuffered (Initial pH 5·5). —●— Buffered initially to pH 6·0.

Extractions of degradation product(s). Oxidised solutions, originally containing 0·5 mg./ml. of apomorphine hydrochloride in 0·9 per cent sodium chloride, were extracted with chloroform and the chloroform phase washed several times with N hydrochloric acid. The blue-black chloroform fraction was then washed repeatedly with water, dried over anhydrous sodium sulphate and the solvent removed under reduced

pressure as recommended by Kaul and others (1961d). The chloroform soluble residue* was suspended in water containing 2 per cent methylcellulose (1,500 cps). This suspension was intraperitoneally administered to pigeons in varying doses and the birds were observed for the following 6 hr.

Results and Discussion

The alterations in concentration of apomorphine hydrochloride and the corresponding changes in biological activity of the preparations with time are graphically presented in Figs. 1 and 2. The higher pH favours more rapid degradation as has been demonstrated by Veit (1935) and Kaul and others (1959).

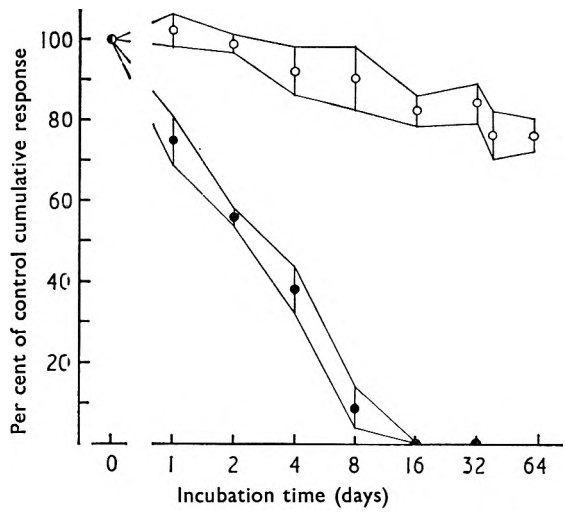


FIG. 2. Biological activity of degrading apomorphine HCl in pigeons. Original concentration: 0·5 mg. per ml. of 0·9 per cent sodium chloride solution. Points represent mean Cumulative Pecking Response ± standard error in terms of per cent of control. —○— Unbuffered (Initial pH 5·5). —●— Buffered initially to pH 6·0.

During the incubation period a gradual reduction in pH of all degrading solutions was noted. The unbuffered preparations had an initial reaction of pH 5·5 which progressively decreased to pH 3·6 after 61 days. Other preparations, buffered initially to pH 6·0, had a reaction of pH 4·6 after 32 days, the most rapid decrease occurring during the last 16 days.

The change in intensity of the pecking syndrome that accompanies the ageing of unbuffered incubated solutions is far from striking and reflects the very gradual inactivation of apomorphine. At the end of 61 days incubation, approximately 70 per cent of the original apomorphine was still present and this was sufficient to initiate a pecking syndrome that was 75 per cent as intense as that produced by the original solution. The

* 50 mg. of apomorphine hydrochloride (42·7 mg. apomorphine base) yielded 25 mg. of chloroform soluble residue.

solution, at this point, was dark green in colour with black precipitate detectable on the floor and walls of the container. Visual inspection entirely fails to give the observer a basis for estimating the degree of decomposition. The very rapidly developing green colour of a freshly prepared unbuffered solution of apomorphine hydrochloride deceptively impresses one with what is interpreted to be a high rate of degradation. It came perhaps as a surprise to Gorrell and Gray (1928) and others (Corbelli, 1911; Ponte, 1935) to discover that although their aged apomorphine preparations were extremely discoloured, they nevertheless still retained emetic activity. On the other hand, there have been reports of complete loss of emetic activity upon storage for 6 months, after sterilising in a water-bath (Laurino, 1936) and after incubation with rabbit serum for 2 hr. (Takahashi, 1934). In none of the studies cited was a quantitative analysis performed and we therefore have no information about the actual state of decomposition. These reports on biological activity changes have been based entirely upon an emetic end-point, a response which is difficult to make quantitative. Thus, statements reflect extremes only—retention or loss of emetic activity with no indication that there may have been a partial loss. Emesis simply does not lend itself readily to a graded quantitative analysis.

Our experience with the apomorphine-induced pecking syndrome has served to provide us with a readily measurable index of apomorphine activity. Initial explorations with aged unbuffered solutions seemed to support the observations of those investigators who failed to detect a loss of apomorphine potency in spite of the fact that the solutions were extremely discoloured. This suggested that either the degradation products possess the same activity as the parent apomorphine or that the intense discoloration may actually be due to the degradation of only minute amounts of apomorphine. The results presented here support the latter explanation. The more rapidly oxidising solutions (buffered initially at pH 6·0) lost activity much faster than unbuffered solutions. In both types of preparations the loss in biological activity closely paralleled the disappearance of unoxidised apomorphine.

Changes in the concentration of apomorphine in the unbuffered solutions occurring during the first 2 days were too small to be detected by the biological method hence changes in activity here are not significant. On the other hand, after 8 days incubation no activity at all could be detected in the buffered preparations since the residual concentration of apomorphine at this time was apparently subthreshold (Burkman, 1950). For these reasons the curves for biological activity cannot be perfectly superimposed over those measuring apomorphine concentration. Within the range of maximal biological sensitivity, however, the two assays are in excellent agreement.

The chloroform-soluble component of the degradation mixture (which represented approximately 45 per cent of the total oxidation product) was administered to a total of 24 pigeons in doses ranging from 0·5 mg./kg. to 10 mg./kg., i.p. All doses failed to evoke a pecking response. The material was, in fact, devoid of all gross behavioural effect.

REFERENCES

Burkman, A. M. (1960). *J. Amer. pharm. Ass., Sci. Ed.*, **49**, 558–559.
Burkman, A. M. (1961a). *J. pharm. Sci.*, **50**, 156–160.
Burkman, A. M. (1961b). *Ibid.*, **50**, 771–773.
Burkman, A. M. (1962). *Arch. int. Pharmacodyn.*, **137**, 396–403.
Corbelli, U. (1911). *Boll. Chim. Farm.*, **50**, 871–873.
Gorrell, J. E. and Gray, P. L. (1928). *Proc. Soc. exp. Biol. N.Y.*, **25**, 619–622.
Kaul, P. N., Brochmann-Hanssen, E. and Way, E. L. (1959). *J. Amer. pharm. Ass., Sci. Ed.*, **48**, 638–641.
Kaul, P. N., Brochmann-Hanssen, E. and Way, E. L. (1961a). *J. pharm. Sci.*, **50**, 244–247.
Kaul, P. N., Brochmann-Hanssen, E. and Way, E. L. (1961b). *Ibid.*, **50**, 248–251.
Kaul, P. N., Brochmann-Hanssen, E. and Way, E. L. (1961c). *Ibid.*, **50**, 840–842.
Kaul, P. N., Brochmann-Hanssen, E. and Way, E. L. (1961d). *Ibid.*, **50**, 266–267.
Laurino, L. F. (1936). *Rev. Facultad Agron. Vet. Univ. Buenos Aires*, **8**, 323–325.
McIlvaine, T. C. (1921). *J. biol. Chem.*, **49**, 183–186.
Ponte, D. (1935). *Giorn. Farm. Chim.*, **84**, 53–57.
Takahashi, S. (1934). *Arb. Dritt. Abt. Anat. Inst. Kaiser. Univ. Kyoto*, Series D, No. 4, p. 138–139.
Veit, F. (1935). *Arch. exp. Path. Pharmak.*, **178**, 577–592.

POSTURAL DRAINAGE OF RESPIRATORY TRACT FLUID IN PHOSGENE-INDUCED PULMONARY OEDEMA

BY ELDON M. BOYD AND WILLIAM F. PERRY*

From the Department of Pharmacology, Queen's University, Kingston, Ontario, Canada

Received February 6, 1963

Cats and dogs were given phosgene by inhalation at a dose in the range of the LD50. During the hour or two before death, the volume output of respiratory tract fluid increased some 30-fold in half the animals and its lipid and sodium chloride contents were similar to those of blood plasma. Postural pulmonary drainage did not augment the volume output of respiratory tract fluid nor consistently affect chemical or histological measurements upon the lungs and chemical measurements upon respiratory tract fluid and blood. Postural pulmonary drainage did not increase the survival rate of intact rats exposed to phosgene. The ability of the animals to excrete such large volumes of respiratory tract fluid appeared to be due to a marked reserve capacity of the ciliary drainage mechanism which was evidently not affected by the dose of phosgene given to these animals.

IN a previous communication Boyd and Perry (1960) reported a sixty fold increase in the volume output of respiratory tract fluid just before death in the late symptomatic period of pulmonary oedema induced by inhalation of phosgene in rabbits. The sodium, chloride, and lipid levels of this respiratory tract fluid were insignificantly different from those of the animal's blood plasma. It appeared possible that the presence of such huge amounts of plasma-like fluid might tax the capacity of the lungs to eliminate it and that death might be the result of failure to remove the fluid from the respiratory airway. It was found that postural pulmonary drainage did not augment the output of respiratory tract fluid in cats or dogs treated with phosgene nor increase the survival rate in albino rats so treated. The results indicate that the ciliary mechanism for the elimination of respiratory tract fluid has tremendous reserve capacity.

METHODS

Techniques employed were similar to those reported by Boyd and Perry (1960). The animals were exposed to an estimated LD50 of phosgene (Spector, 1956) by the static method in a chamber of 400 litres capacity. The initial concentration of phosgene was varied between 0·10 and 0·30 mg./litre, depending upon the relative humidity of the laboratory air and the number and weight of animals exposed at one time. The half life of phosgene varied with the relative humidity of air inside the sealed chamber; at 25 per cent relative humidity, for example, the half life of these concentrations of phosgene averaged 10 min. The exposure time was 30 min. Identical exposures were used for each group of animals subsequently divided into equal numbers and posturally drained at angles of 0°, 30°, or 50° with the horizontal.

* Present address: Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada.

Experiments were made upon 57 cats exposed to phosgene and upon 12 controls not exposed. At intervals of up to 17 hr. after exposure, they were anaesthetized with urethane and arranged for the collection of respiratory tract fluid after the technique of Boyd (1954). Equal numbers of animals were assembled, for this purpose, on tilt tables with the long axis of the body held, head downward, at angles of 0° (prone upon the belly), 30°, and 50° with the horizontal. Similar experiments were made upon 24 dogs, half exposed and half not exposed to phosgene. One hundred and twenty-six albino rats, with 30 controls, were similarly treated except that they were not operated upon for the collection of respiratory tract fluid but rather maintained in straight jackets under sedation with urethane at the same angles of postural pulmonary drainage.

The observations and measurements noted below were made by methods described by Boyd and Perry (1960). Statistical methods were those of Croxton (1953).

Results

Exposure to phosgene increased the output of respiratory tract fluid, particularly in the hour or two before death. Postural pulmonary drainage did not produce a further increase in the volume output of this fluid as shown by results summarized in Fig. 1. Not included in data averaged in Fig. 1 are two cats arranged in the prone (0°) position in which, during the hour before death, the volume output of respiratory tract fluid reached values 1½ and 2½ thousand-fold the normal output. In confirmation of

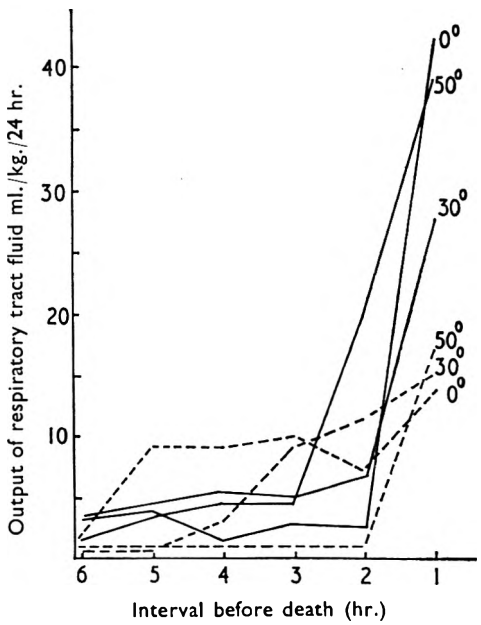


FIG. 1. The mean volume output of respiratory tract fluid in cats (solid lines) and dogs (broken lines) exposed to phosgene and arranged for postural pulmonary drainage at angles of 0°, 30° and 50° with the horizontal.

the findings of Boyd and Ronan (1942), postural pulmonary drainage also had no effect upon the volume output of respiratory tract fluid in control cats and dogs not exposed to phosgene.

The mean ± standard deviation hours survival during which respiratory tract fluid was collected was 37·1 ± 13·9 in control cats and 8·1 ±3·8 in cats exposed to phosgene. Corresponding figures in dogs were 20·5 ± 12·2 and 9·8 ± 7·3. In neither cats nor dogs was survival time related to the angle of postural pulmonary drainage. In albino rats exposed to phosgene but not arranged for collection of respiratory tract fluid, there were 23 per cent survivors at 24 hr. in animals held at 0°, 17 per cent at 30°, and 30 per cent at 50° with no deaths at corresponding angles in the controls not given phosgene. No evidence was obtained, therefore, that postural pulmonary drainage increased the rate of survival of animals exposed to lethal doses of phosgene.

TABLE I

THE HISTOPATHOLOGY OF THE LUNGS IN CATS AND DOGS AT DEATH FROM PHOSGENE INHALATION*

Measurement	Angle of drainage		
	0°	30°	50°
Cats			
Oedema	2·8 ± 1·0	2·3 ± 1·0	2·5 ± 1·2
Congestion	1·4 ± 1·4	0·7 ± 1·2	1·6 ± 1·5
Emphysema	2·0 ± 1·1	1·9 ± 1·6	1·0 ± 1·1†
Atelectasis	0·0 ± 0·0	0·1 ± 0·3	0·4 ± 0·7
Haemorrhage	0·6 ± 0·8	0·2 ± 0·6	0·7 ± 0·8
Contracted arteries	0·4 ± 0·7	0·5 ± 1·2	0·5 ± 1·0
Dogs			
Oedema	4·0 ± 0·0	1·5 ± 0·5†	
Congestion	4·0 ± 0·0	4·0 ± 0·1	
Emphysema	2·0 ± 0·5	2·5 ± 1·5	
Atelectasis	0·0 ± 0·0	0·0 ± 0·0	
Haemorrhage	3·0 ± 1·0	3·0 ± 1·0	
Contracted arteries	0·0 ± 0·0	0·0 ± 0·0	

* The results are expressed as mean ± standard deviation arbitrary (1+ to 4−) units. Means in animals drained at 30° or 50° which differed at P = 0·05 or less from means in animals drained at 0°, by a *t* test, are indicated thus: †.

As shown by results summarized in Table I, postural pulmonary drainage had no consistent effect upon the histopathologic appearance of the lungs at autopsy. The lumen of the trachea, bronchi, and bronchioles often contained an exudate but the lining mucosa appeared normal.

Postural pulmonary drainage had no consistently significant effect upon the sodium, chloride, and lipid content of respiratory tract fluid in control animals or in animals exposed to phosgene. Data supporting this conclusion are presented in Table II. In over half of the animals there occurred a pre-mortem gush or marked increase in the output of respiratory tract fluid. This group corresponded to the rabbits of Group III in the report of Boyd and Perry (1960). The incidence of all animals with a pre-mortem gush was 55 per cent in animals held at 0°, 52 per cent at 30°, and 62 per cent at 50°. In animals with a pre-mortem gush, the composition of respiratory tract fluid was almost identical to that of blood plasma.

TABLE II

MEASUREMENTS UPON RESPIRATORY TRACT FLUID*

Measurement	Controls	Phosgene-treated; angle of drainage		
		0°	30°	50°
Cats				
Sodium	59 ± 35	219 ± 244	159 ± 120†	175 ± 106†
Chloride	74 ± 44	280 ± 151†	190 ± 163†	270 ± 119†
Total lipid	60 ± 4	221 ± 119†	272 ± 69†	155 ± 61†
Neutral fat	12 ± 7	52 ± 51†	103 ± 84†	62 ± 56†
Total fatty acids	33 ± 8	130 ± 74†	180 ± 56†	104 ± 54†
Total cholesterol	19 ± 8	61 ± 29†	60 ± 36†	32 ± 11†S
Ester cholesterol	12 ± 2	39 ± 26†	40 ± 23†	20 ± 9†
Free cholesterol	7 ± 5	22 ± 14†	20 ± 8†	12 ± 4†
Phospholipid	22 ± 10	82 ± 73†	81 ± 51†	48 ± 23†
Dogs				
Sodium	44 ± 18	338 ± 18†	334 ± 23†	
Chloride	59 ± 21	381 ± 16†	352 ± 57†	
Total lipid	197 ± 49	332 ± 41†	279 ± 62	
Neutral fat	53 ± 31	50 ± 38	91 ± 23	
Total fatty acids	126 ± 39	208 ± 33†	180 ± 42	
Total cholesterol	41 ± 15	53 ± 11	63 ± 10†	
Ester cholesterol	28 ± 19	36 ± 16	46 ± 7	
Free cholesterol	13 ± 4	17 ± 4	17 ± 3	
Phospholipid	85 ± 37	205 ± 18†	95 ± 35	

* The results are expressed as mean ± standard deviation mg./100 ml. of respiratory tract fluid. Means in phosgene-treated animals which differed at P = 0·05 or less from means in controls, by a *t* test, are indicated thus: †. Means in phosgene-treated animals drained at 30° or 50° which correspondingly differed from means in phosgene-treated animals drained at 0° are indicated by S.

TABLE III

MEASUREMENTS UPON BLOOD PLASMA*

Measurement	Units	Controls	Phosgene-treated; angle of drainage		
			0°	30°	50°
Cats					
Sodium	mg.	280 ± 20	276 ± 35	294 ± 16	291 ± 14
Chloride	mg.	353 ± 26	368 ± 58	391 ± 24	385 ± 29
Total lipid	mg.	392 ± 56	342 ± 81	334 ± 90	391 ± 117
Neutral fat	mg.	86 ± 76	69 ± 63	88 ± 54	73 ± 44
Total fatty acids	mg.	242 ± 74	200 ± 59	203 ± 63	226 ± 61
Total cholesterol	mg.	92 ± 31	88 ± 40	85 ± 27	105 ± 51
Ester cholesterol	mg.	58 ± 29	51 ± 29	52 ± 20	66 ± 39
Free cholesterol	mg.	34 ± 12	37 ± 19	33 ± 12	39 ± 15
Phospholipid	mg.	167 ± 41	151 ± 25	126 ± 51†	169 ± 70
Haemoglobin	g.	11·5 ± 1·9	13·9 ± 3·3†	13·8 ± 2·6†	14·1 ± 4·0
Haematocrit	ml.	31·2 ± 3·6	38·3 ± 9·5†	33·8 ± 6·7	41·4 ± 11·5†
Dogs					
Sodium	mg.	283 ± 24	262 ± 35	288 ± 35	
Chloride	mg.	350 ± 10	353 ± 9	369 ± 19	
Total lipid	mg.	590 ± 152	430 ± 83†	616 ± 150	
Neutral fat	mg.	182 ± 74	94 ± 22†	136 ± 69	
Total fatty acids	mg.	386 ± 105	273 ± 60†	369 ± 99	
Total cholesterol	mg.	110 ± 29	77 ± 7†	144 ± 30S	
Ester cholesterol	mg.	59 ± 19	48 ± 10	69 ± 28	
Free cholesterol	mg.	51 ± 19	29 ± 4†	75 ± 29S	
Phospholipid	mg.	258 ± 78	227 ± 47	291 ± 94	
Haemoglobin	g.	14·5 ± 0·5	29·3 ± 8·8†	16·3 ± 6·9	
Haematocrit	ml.	37·0 ± 3·0	75·0 ± 14·0†	45·3 ± 17·6	

* The results are expressed as mean ± standard deviation units/100 ml. of blood plasma, excepting haemoglobin and haematocrit which are per 100 ml. of whole blood. Means in phosgene-treated animals which differed at P = 0·05 or less from means in controls, by a *t* test, are indicated thus: †. Means in phosgene-treated animals drained at 30° or 50° which correspondingly differed from means in phosgene-treated animals drained at 0° are indicated by S.

Measurements upon blood and blood plasma are summarized in Table III. In phosgene poisoning there occurred an increase in the hematocrit and in the concentration of haemoglobin. When this was marked, as in dogs held at 0° angles, the concentration of plasma lipids was sometimes reduced. Postural pulmonary drainage did not consistently affect the results.

TABLE IV

MEASUREMENTS UPON THE LUNGS AND TRACHEA*

Measurement	Units	Controls	Phosgene-treated; angle of drainage		
			0°	30°	50°
Cats					
Periphery, water	g./100 g. wet wt.	77·7 ± 1·10	86·1 ± 2·6†	85·3 ± 2·4†	84·2 ± 2·0†
Periphery, chloride	mg./g. dry wt.	10·6 ± 1·4	19·9 ± 2·8†	18·5 ± 3·1†	17·4 ± 2·9†S
Periphery, iron	µg./g. dry wt.	561 ± 65	483 ± 232	496 ± 198	503 ± 162
Hilus, water	g./100 g. wet wt.	76·5 ± 1·8	85·2 ± 2·2†	84·1 ± 2·7†	83·3 ± 2·5†
Hilus, iron	µg./g. dry wt.	425 ± 42	370 ± 169	472 ± 169	423 ± 165
Trachea, water	g./100 g. wet wt.	69·5 ± 2·9	73·1 ± 4·5†	75·2 ± 4·0†	70·5 ± 4·3
Trachea, iron	µg./g. dry wt.	124 ± 43	112 ± 62	121 ± 48	125 ± 40
Dogs					
Periphery, water	g./100 g. wet wt.	78·7 ± 0·9	84·3 ± 0·3†	81·7 ± 2·8	
Periphery, chloride	mg./g. dry wt.	9·4 ± 0·8	16·9 ± 1·5†	12·4 ± 3·3S	
Periphery, iron	µg./g. dry wt.	852 ± 176	789 ± 29	648 ± 154	
Hilus, water	g./100 g. wet wt.	77·3 ± 1·3	82·5 ± 1·4†	80·5 ± 2·7	
Hilus, iron	µg./g. dry wt.	723 ± 302	737 ± 63	640 ± 68	
Trachea, water	g./100 g. wet wt.	69·9 ± 3·0	69·6 ± 1·8	70·0 ± 1·8	
Trachea, iron	µg./g. dry wt.	104 ± 91	100 ± 20	88 ± 10	
Albino Rats					
Lung, water	g./100 g. wet wt.	79·4 ± 2·8	83·2 ± 3·7†	83·2 ± 1·5†	84·0 ± 1·5†
Lung, chloride	mg./g. dry wt.	10·5 ± 2·0	15·1 ± 1·8†	12·4 ± 3·0	13·4 ± 1·6†
Lung, iron	µg./g. dry wt.	864 ± 327	698 ± 131†	548 ± 261†	772 ± 215

* The results are expressed as mean ± standard deviation. Means in phosgene-treated animals which differed at $P = 0{\cdot}05$ or less from means in controls, by a t test, are indicated thus: †. Means in phosgene-treated animals drained at 30° or 50° which correspondingly differed from means in phosgene-treated animals drained at 0° are indicated by S.

Finally, measurements upon the lung periphery, hilus, and trachea are summarised in Table IV. In phosgene-induced pulmonary oedema, levels of water and chloride were consistently increased while levels of iron tended to decrease. There was some evidence of a tendency for these changes to be less marked in animals arranged at 30° and 50° angles. In a separate experiment, it was found that control albino rats held at these angles for 72 hr. had lower levels of whole lung water and chloride and higher levels of iron than had albino rats held at a 0° angle. It may be concluded, therefore, that changes in the chemical composition of the lungs induced by inhalation of phosgene were not affected by postural pulmonary drainage.

DISCUSSION

Insofar as indicated by the measurements used, the course of phosgene intoxication in cats, dogs, and rats was essentially similar to that in rabbits reported by Boyd and Perry (1960). In half of the cats and dogs, there occurred a pre-mortem gush or marked increase in the volume output of

respiratory tract fluid. The sodium, chloride, and lipid contents of this gushed respiratory tract fluid were similar to those of blood plasma. Cameron and Courtice (1946) found the protein content of respiratory tract fluid drained from the lungs after death from phosgene to be similar to that of blood plasma in rabbits, dogs, and goats.

At death, the alveolar portions of the lungs were oedematous, congested, and emphysematous, with areas of atelectasis, hemorrhage, and contracted arteries and with an increase in the levels of water and chloride. The trachea and bronchi were normal in microscopic appearance. The hematocrit and concentration of blood haemoglobin were increased, particularly in dogs in which plasma lipid content was sometimes depressed. Cameron and Courtice (1946) reported a fall in plasma protein concentration in dogs exposed to phosgene. While plasma transfusions temporarily relieve the haemoconcentration, they increase the pulmonary oedema and are detrimental (Courtice and Foss, 1946).

Postural pulmonary drainage did not augment the output of respiratory tract fluid nor survival rate and had no consistent effect upon other measured signs of phosgene poisoning in the animals used. During the pre-mortem gush, the volume output of respiratory tract fluid averaged some thirty fold the normal output, indicating a very high reserve capacity of the lungs to excrete respiratory tract fluid. The most important single mechanism for the excretion of respiratory tract fluid appears to be ciliary action (Boyd, 1954). There was no histological evidence of damage to the cilia of the bronchi and trachea in the cats and dogs used in this study of phosgene intoxication. When the cilia are destroyed by inhalation of ammonia gas or steam, excretion is retarded and the output of respiratory tract fluid is increased by postural pulmonary drainage (Boyd, Perry and Stevens, 1944).

Acknowledgement. The authors wish to acknowledge the assistance of G. H. Ettinger, J. Gastle, M. L. MacLachlan, M. A. Price-Jones, E. P. Sheppard, M. E. T. Stevens, and W. C. Stewart.

References

Boyd, E. M. (1954). *Pharmacol. Rev.*, **6**, 521–542.
Boyd, E. M. and Perry, W. F. (1960). *J. Pharm. Pharmacol.*, **12**, 726–732.
Boyd, E. M., Perry, W. F. and Stevens, M. E. T. (1944). *Amer. J. Physiol.*, **140**, 467–473.
Boyd, E. M. and Ronan, A. (1942). *Ibid.*, **135**, 383–386.
Cameron, S. R. and Courtice, F. C. (1946). *J. Physiol.*, **105**, 175–185.
Courtice, F. C. and Foss, G. L. (1946). *Lancet*, **2**, 670–671.
Croxton, F. E. (1953). *Elementary Statistics with Applications in Medicine*, New York: Prentice-Hall Inc.
Spector, W. S. (1956). *Handbook of Toxicology*, Volume I, *Acute Toxicities of Solids, Liquids and Gases to Laboratory Animals*, Philadelphia: W. B. Saunders Company.

AMPHETAMINE-LIKE ACTIVITY OF β-PHENETHYLAMINE AFTER A MONOAMINE OXIDASE INHIBITOR *IN VIVO*

By P. Mantegazza and M. Riva

From the Department of Pharmacology, School of Medicine, University of Milan, Milan, Italy

Received January 28, 1963

β-Phenethylamine possesses marked amphetamine-like effects which are demonstrable in animals pre-treated with a monoamine oxidase inhibitor. Like amphetamine, β-phenethylamine induces an increase of coordinated spontaneous motility in mice, anorexia in rats and dogs, hyperthermia in mice and rats, and exhibits a difference in lethality between isolated and aggregated mice. These effects are seen with similar doses of β-phenethylamine or amphetamine. But, unlike amphetamine, β-phenethylamine does not increase coordinated spontaneous motility in rats.

β-Phenylethylamine has weak sympathomimetic activities (Bovet and Bovet-Nitti, 1948) which can best be demonstrated in animals pretreated with a monoamine oxidase inhibitor (Griesemer, Barsky, Dragsted, Wells and Zeller, 1954; Rebhun, Feinberg and Zeller, 1954; Bachtold and Pletscher, 1957). Under these conditions the amine can also induce amphetamine-like symptoms of central stimulation.

The present experiments were designed to evaluate the amphetamine-like effects of β-phenethylamine on a quantitative basis, by measuring some activities considered to be characteristic of amphetamine. Enhanced spontaneous coordinated motility, anorexigenic effect, hyperthermic activity and increased mortality in aggregated situations have been investigated.

Methods

Animals. The animals used were adult mongrel dogs, Wistar rats weighing 100 to 200 g. and Swiss mice weighing 18 to 20 g. Anorexigenic activity was studied in male rats and in dogs of both sexes. Female rats and mice were used in the study of locomotor activity and hyperthermia. During experiments the animals were kept in a semi-dark and quiet room, the temperature of which was maintained from 20 to 22°.

Drugs. Iproniazid phosphate (Hoffmann La Roche) was used as a monoamine oxidase inhibitor. Subcutaneous treatment with iproniazid was given 24 hr. before the injection of β-phenethylamine hydrochloride at a dose of 200 mg./kg. (rats and mice) or 129 mg./kg. (dogs). These doses produce long, intensive inhibition of monoamine oxidases.

(±)-Amphetamine sulphate (Recordati) and β-phenethylamine hydrochloride (Hoffmann La Roche) were used. These compounds were administered subcutaneously over a range of doses to allow accurate representation of the activity pattern. An isotonic solution of sodium chloride was used for control injection.

Hyperthermia. Groups of six rats or mice were fasted for 12 hr. and were placed in cages measuring 45 × 40 cm. Rectal temperature was

measured hourly during five successive hr. by means of an Electric Universal Thermometer of the TE2 Ellab type. Amphetamine or phenethylamine was injected after the basal temperature had been taken twice. Animals which had an abnormal basal temperature were discarded.

Spontaneous activity. The method used was similar to that described by Dews (1953) for the study of spontaneous coordinated motility in mice.

Five mice or 4 rats were used at the same time in each experiment. The cages for mice measured 20 × 30 cm., and those for rats 45 × 30 cm. To avoid disturbing effects, infra-red photo-cells were used and the recording counters were installed in an adjacent room. Animals were injected subcutaneously 30 min. before being placed in the activity cages, spontaneous motility was recorded for a 15 min. period. Control experiments were made at random during the series of tests. At least 20 animals were used at each dose level.

Anorexigenic activity in rats. Animals were trained to take food during 8 hr. out of 24. They were kept in individual cages and generally developed a consistent habit of food intake within two weeks. On the day of the experiment a weighed meal was given to each animal immediately after treatment, and the amount of food intake was registered hourly for 4 hr.

Anorexigenic activity in dogs. A technique similar to that described by Di Ferrante and Longo (1953) was employed. Fifteen dogs were trained to eat a standard meal during a single 30 min. period every day at the same time in the morning. The drugs were injected an hr. before the feeding time. The meal was then offered hourly in order to estimate the presence and duration of the anorexia. The anorexigenic action of drugs was evaluated by considering only those dogs which had refused meals.

Toxicity. Mice and rats were aggregated in groups of 10 in cages measuring 15 × 40 cm. (mice) or 25 × 40 cm. (rats). Comparative studies in isolated conditions were made by placing animals in individual cages. Drugs were injected subcutaneously and mortality was recorded 24 hr. later. At least 10 animals were used at each dose level. The calculations were made according to the method of Litchfield and Wilcoxon (1949).

TABLE I

ANOREXIGENIC ACTIVITY OF PHENETHYLAMINE HYDROCHLORIDE IN NORMAL OR IPRONIAZID PRE-TREATED RATS

Number of animals	Iproniazid (24 hr. before) mg./kg./s.c.	Phenethylamine mg./kg./s.c.	Mean average food intake ± s.e. after injection			
			1 hr.	2 hr.	3 hr.	4 hr.
10	Controls		6·7 ± 0·66	9·0 ± 0·11	10·6 ± 0·09	12·4 ± 0·12
10	—	10	6·1 ± 0·58	8·6 ± 0·61	9·7 ± 0·66	12·5 ± 0·10
34	200	—	5·2 ± 0·40	6·6 ± 0·40	8·1 ± 0·44	9·5 ± 0·56
5	200	5	0·2 ± 0·19	2·0 ± 0·99	3·4 ± 0·99	4·2 ± 1·0
8	200	2·5	0·6 ± 0·37	4·1 ± 0·54	5·5 ± 0·97	6·3 ± 1·0
8	200	1·25	1·8 ± 0·58	4·7 ± 0·84	6·2 ± 0·70	8·1 ± 0·43
8	200	0·62	2·8 ± 0·81	5·2 ± 0·99	7·1 ± 0·76	9·8 ± 0·84

RESULTS

Hyperthermia. Phenethylamine caused hyperthermia only in animals pre-treated with iproniazid (Fig. 1 and 2) and was more active in rats than in mice. In rats phenethylamine produced greater hyperthermia than did amphetamine. In mice this effect was short-lasting and was followed by hypothermia.

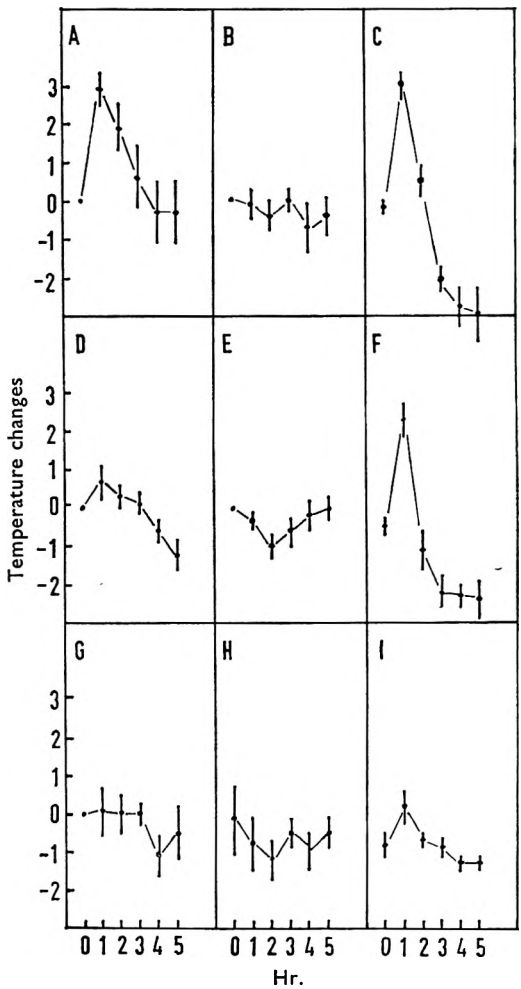


FIG. 1. Hyperthermic effect in mice. Each point on the curves represents the mean temperature of 12–18 mice. Vertical lines indicate the standard error. A, D, G, Amphetamine (10, 5, 2·5 mg./kg., s.c. respectively). C, F, I, Iproniazid (200 mg./kg., s.c.) 24 hr. before phenethylamine (40, 20, 10 mg./kg., s.c. respectively). B. Phenethylamine (40 mg./kg., s.c.), E. No treatment. H. Iproniazid (200 mg./kg., s.c.) 24 hr. before beginning temperature readings.

Spontaneous motility. Phenethylamine produced an increase of coordinated spontaneous motility in mice pre-treated with iproniazid

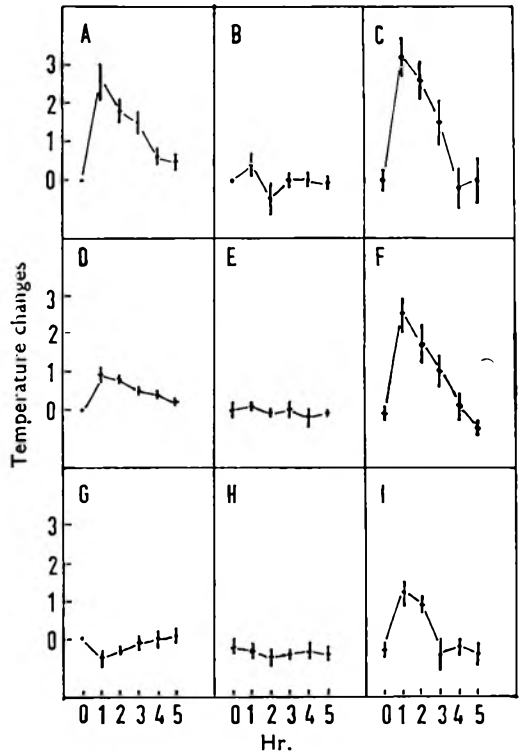


FIG. 2. Hyperthermic effect in rats. Each point on the curves represents the mean temperature of 12–18 rats. Vertical lines indicate the standard error. A, D, G, Amphetamine (10, 5, 2·5 mg./kg., s.c. respectively). C, F, I, Iproniazid (200 mg./kg., s.c.) 24 hr. before phenethylamine (10, 5, 2·5 mg./kg., s.c. respectively). B. Phenethylamine (50 mg./kg., s.c.). E. No treatment. H. Iproniazid (200 mg./kg., s.c.) 24 hr. before beginning temperature readings.

and showed a dose-effect relationship similar to that seen with amphetamine (Fig. 3). This effect was not observed, however, in rats. In this species only symptoms of excitement were evident without any increase of co-ordinated motility. This unexpected negative finding was verified repeatedly at doses of phenethylamine ranging from 1 to 50 mg./kg.

Anorexia. Phenethylamine caused anorexia in animals pretreated with iproniazid; it was more active than amphetamine in rats (Tables I–II),

TABLE II

ANOREXIGENIC ACTIVITY OF AMPHETAMINE SULPHATE IN RATS

Number of animals	Amphetamine mg./kg./s.c.	Mean average food intake ± s.e. after injection			
		1 hr.	2 hr.	3 hr.	4 hr.
49	Controls	6·2 ± 0·35	7·5 ± 0·37	8·9 ± 0·34	10·4 ± 0·42
9	5	1·5 ± 0·58	2·5 ± 0·60	3·0 ± 0·57	5·2 ± 0·93
13	2·5	2·3 ± 0·36	3·6 ± 0·88	6·0 ± 0·61	7·3 ± 0·48
13	1·25	3·0 ± 0·42	4·0 ± 0·69	6·0 ± 0·81	8·1 ± 1·06
13	0·62	4·0 ± 0·61	6·0 ± 0·61	8·0 ± 0·85	10·0 ± 0·96

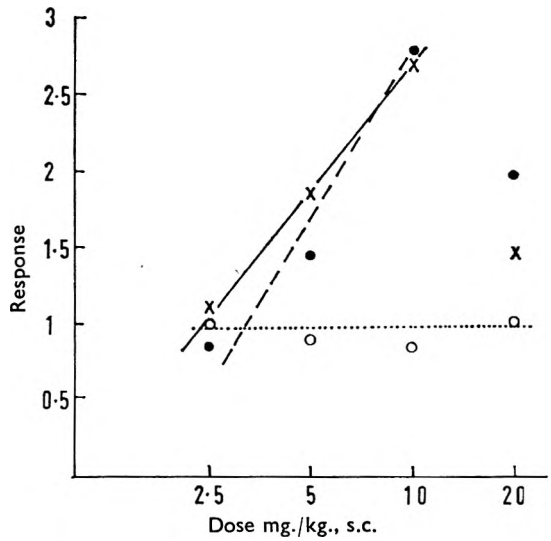


FIG. 3. The effect of phenethylamine hydrochloride on the coordinated activity of normal (○ ····· ○) and of iproniazid pretreated mice (● — — ●) and the effect of amphetamine sulphate on the coordinated activity of normal mice (X——X). Ordinated response expressed as ratio of count after drug to count of controls on same day.

less active than amphetamine in dogs when in animals pretreated with iproniazid, 129 mg/.kg. s.c., phenethylamine, 1–1·69 mg./kg. s.c., delayed food intake 2–10 hr. while amphetamine alone, 1–1·69 mg./kg. s.c., delayed it 3–20 hr.

TABLE III

LETHALITY OF PHENETHYLAMINE HYDROCHLORIDE IN NORMAL OR IPRONIAZID PRETREATED MICE IN AGGREGATED OR ISOLATED SITUATIONS

Iproniazid (24 hr. before) mg./kg./s.c.	Isolated		Aggregated		Increased toxicity potency ratio
	LD50 mg./kg./s.c.	Slope	LD50 mg./kg./s.c.	Slope	
—	420 (280–630)	1·7 (1·0–2·89)	420 (271–651)	1·85 (1·02–3·33)	Not significant
200	290 (250–336)	1·26 (1·06–1·48)	28 (14–53)	2·86 (1·68–4·86)	10·35 (5·4–19·9)
Increased toxicity potency ratio	Not significant		15 (6·9–32·2)		

Toxicity. The data obtained are summarised in Tables III–V. The LD50 of phenethylamine alone was the same in both isolated and aggregated mice and was not significantly different in isolated and aggregated rats. Toxicity increased after treatment with iproniazid. The enhancement was not significant in isolated mice but consisted of a 15-fold increase in aggregated animals. The increase of toxicity in rats was 32-fold in isolated, and 52-fold in aggregated animals. The enhancement of

TABLE IV

LETHALITY OF PHENETHYLAMINE HYDROCHLORIDE IN NORMAL OR IPRONIAZID PRE-TREATED RATS IN AGGREGATED OR ISOLATED SITUATIONS

Iproniazid (24 hr. before) mg./kg./s.c.	Isolated		Aggregated		Increased toxicity potency ratio
	LD50 mg./kg./s.c.	Slope	LD50 mg./kg./s.c.	Slope	
—	750 (614–915)	1·34 (1·13–1·58)	470 (356–620)	1·57 (1·20–2·04)	Not significant
200	23 (12–41)	2·23 (1·11–4·46)	9 (5·3–15·3)	4·3 (1·16–15·9)	2·5 (1·16–5·60)
Increased Toxicity potency ratio	32·6 (17–60)		52·2 (29–93·9)		

TABLE V

LETHALITY OF AMPHETAMINE SULPHATE IN AGGREGATED OR ISOLATED SITUATIONS

Animals	Isolated		Aggregated		Increased toxicity potency ratio
	LD50 mg./kg./s.c.	Slope	LD50 mg./kg./s.c.	Slope	
Mice	205 (164–256)	1·41 (1·14–1·73)	15·5 (12–20)	1·45 (1·20–1·74)	13·2 (9·4–18·4)
Rats	37 (30–45·5)	1·38 (1·10–1·72)	20·5 (17·6–23·7)	1·27 (0·99–1·62)	1·80 (1·38–2·34)

toxicity of phenethylamine in aggregated animals is therefore more marked in mice than in rats.

The LD50 of amphetamine in both isolated and aggregated mice and in isolated rats was similar to the corresponding LD50 of phenethylamine in mice pre-treated with iproniazid (Table V). No significant increase in toxicity of amphetamine was seen in aggregated rats.

DISCUSSION

The data reported indicate that phenethylamine, in animals pre-treated with iproniazid, and amphetamine are analogous to each other, both qualitatively and quantitatively. Phenethylamine causes anorexia, hyperthermia and enhanced toxicity in aggregated animals. In addition, these effects are induced by doses similar to the doses of amphetamine producing the same effects.

It is known that phenethylamine, in contrast to amphetamine, is rapidly inactivated by monoamine oxidases (Blaschko, 1952). The fact that, after the inhibition of these enzymes, phenethylamine is similar to amphetamine, both qualitatively and quantitatively, constitutes a further indication that the methyl group on the α-carbon atom of the ethylamine side-chain is not essential for amphetamine-like activity. This methyl group, which differentiates the structure of amphetamine from that of phenethylamine, is however important in preventing inactivation of the molecule by monoamine oxidases. Similar conclusions have been reached recently by Van der Schoot, Ariëns, van Rossum and Hurkmans (1962).

As far as the spontaneous co-ordinated motility is concerned, it appears to be increased by phenethylamine, after a monoamine oxidase inhibitor,

in mice, but not in rats. The rats were clearly excited but not in a co-ordinated manner. It is difficult to explain this unexpected negative result since, in rats, the other effects of amphetamine and phenethylamine are strikingly similar.

On the other hand, iproniazid pre-treatment does not alter the experimental conditions necessary for the demonstration of an increased motor activity, since the amphetamine effect alone or after iproniazid treatment, changes only in intensity and not in quality.

It may be supposed that in rats, after monoamine oxidase inhibition, phenethylamine is transformed into a metabolite which interferes with the co-ordinated motor exciting activity. For instance, phenethylamine is known to be transformed into β-hydroxy-β-phenethylamine by dopamine β-oxidase (Pisano, Creveling and Udenfriend, 1960); this compound may interfere with some of the actions of β-phenethylamine. Preliminary results obtained in this laboratory seem to be consistent with this hypothesis.

References

Bachtold, H. and Pletscher, A. (1957). *Experientia*, **15**, 163–165.
Blaschko, H. (1952). *Pharmacol. Rev.*, **4**, 415–458.
Bovet, D. and Bovet-Nitti, F. (1948). *Médicaments du Système Nerveux Végétatif*, pp. 74–77, Bale: S. Karger.
Dews, P. B. (1953). *Brit. J. Pharmacol.*, **8**, 46–48.
Di Ferrante, N. and Longo, V. G. (1953). *Farmaco, Ed. Sci.*, **8**, 16–21.
Griesemer, E. C., Barsky, J., Dragsted, C. A., Wells, J. A. and Zeller, E. A. (1954). *Proc. Soc. exp. Biol., N.Y.*, **84**, 699–701.
Litchfield, J. T. and Wilcoxon, F. (1949). *J. Pharmacol.*, **96**, 99–113.
Pisano, J. J., Creveling, C. R. and Udenfriend, S. (1960). *Biochim. Biophys. Acta*, **43**, 566–568.
Rebhun, J., Feinberg, S. M. and Zeller, E. A. (1954). *Proc. Soc. exp. Biol., N.Y.* **87**, 218–220.
Van der Schoot, J. B., Ariëns, E. J., van Rossum, J. M. and Hurkmans, J. A. Th. M. (1962). *Arzneimit.-Forsch.*, **12**, 902–907.

A NOTE ON THE STABILITY OF OPHTHALMIC SOLUTIONS CONTAINING PILOCARPINE HYDROCHLORIDE ALONE AND WITH ESERINE

By Rune Fagerström

From the Central Military Pharmacy and Department of Pharmacognosy, Kungl. Farmaceutiska Institutet, Stockholm, Sweden

Received January 11, 1963

The stability of pilocarpine hydrochloride in ophthalmic solutions after heating and storage, and the influence of light and of two types of containers on stability, have been investigated. Pilocarpine was assayed by a modification of a published method. In ophthalmic solutions sterilised by heating to 100° for 30 min. it is stable for at least one year at 15–25° in the dark in bottles of good quality glass covered with a nylon cap. The stability of ophthalmic solutions containing pilocarpine and eserine during storage and after different methods of sterilisation was also investigated. This solution can be heated at 100° for 15 min. and, stored in suitable bottles, it is stable for at least 18 months

According to the Swedish Pharmacopoeia, solutions of pilocarpine hydrochloride shall be sterilised by heating at 100° for 1 hr. *The Extra Pharmacopoeia* (1958) recommends sterilisation by autoclaving. According to Riegelman and Vaughan (1958), the substance withstood 24 hr. of autoclaving (120°) with approximately 5 per cent destruction. No reports have been published on its stability in solutions upon protracted storage after sterilisation by heating or by autoclaving.

Eserine is unstable; its greatest stability was found by Hellberg (1949b) to be at pH values of 6 or lower. A 0·4 per cent solution of eserine salicylate in 2 per cent boric acid was stable for 100 days at 25°. Hellberg also considered that eserine solutions could not be sterilised by heating, whatever the pH value. Schradie and Miller (1959) have shown that it is possible to apply a solution with a pH of about 3 without damaging the cornea, if the solution does not have an unduly high buffer capacity. As Hellberg (1949b) indicated that the stability of unheated solutions of eserine might be greater at lower pH values, stability in the pH range 3·5–4·0 has been studied.

Experimental

Effect of Light on the Stability of Pilocarpine Solutions

Two glass-stoppered volumetric flasks were filled with an 0·2 per cent aqueous solution of pilocarpine hydrochloride. One was stored in daylight and the other in the dark for 21 months at 15–25°, after which the pilocarpine content of each was determined.

Effect of Heating on the Stability of Pilocarpine Solutions

An ophthalmic solution of pilocarpine hydrochloride prepared according to the Swedish Pharmacopoeia (Ed. XI), (aqueous solution of 2 per cent pilocarpine hydrochloride with sodium chloride 0·4, and phenyl mercuric nitrate 0·001 per cent) was dispensed in glass ampoules or in

the containers described by Linde (1957). These were of good quality glass with a cap of a polyamide, related to nylon, the top of which is shaped as a tube suitable for giving drops. The cap is closed with a stopper permanently connected to the container.*

Half the containers and ampoules were autoclaved at 110° for 30 min. (Swedish Pharmacopoeia method) and stored in the dark at room temperature for 12 months. The rest were similarly stored without autoclaving.

Pilocarpine content and pH as well as the optical rotation of the solutions were determined before and after heating and storage.

Stability of Pilocarpine and Eserine Solutions

It has been considered possible to stabilise eserine solutions with a reducing agent but, as noted by Hellberg (1949a) and verified by us, the frequently used sodium bisulphite ($NaHSO_3$) causes a low pH, hence it was replaced by ascorbic acid. The ophthalmic solution studied had the composition: pilocarpine hydrochloride, 2; eserine salicylate, 0·2; sodium chloride, 0·1; ascorbic acid, 0·1 g.; sterilised water to 100 ml. It was dispensed in ampoules and the containers described by Linde (1957) and was sterilised by filtration or heating at 100° for 15 min. The content of pilocarpine and eserine was determined immediately and 18 months after sterilisation.

The storage conditions were: (i) in a refrigerator at 4–6°; (ii) in a dark room at 12–15°; (iii) protected from light in a place where the temperature varied with the outdoor temperature from about −10 to +30°.

Assay of Pilocarpine

Pilocarpine can be determined photometrically as the perchromate, a blue compound formed by treating a slightly acid solution of pilocarpine with potassium dichromate and hydrogen peroxide. The method adopted was a modification of that of Levine and Horrocks (1960).

The sample (2·00 ml., equivalent to about 4 mg. of pilocarpine hydrochloride) is pipetted into a 125 ml. separating funnel. 20 per cent acetic acid AR (1·0 ml.), chloroform AR (10 ml.) and 5 per cent potassium chromate AR (1 ml.) is added and then, rapidly, 3 per cent hydrogen peroxide AR (2·0 ml.) by syringe. The funnel is immediately shaken vigorously for 90 sec. The chloroform phase is filtered into a 25 ml. volumetric flask. The extraction is repeated with chloroform (10 and then 5 ml.) and the filter is washed with small portions of chloroform, which are added to the combined chloroform extracts until the flask is made up to the mark. The solutions are protected from light before measuring. The extinction is determined within 20 min. at 560 mμ and the corresponding amount of pilocarpine read from a standard curve prepared from pilocapine solutions of known content. A straight line passing through the origin was obtained within the sample range 0·2–0·7 mg. of pilocarpine.

This procedure is also suitable in presence of eserine.

* "Sonyl" bottle.

Assay of Eserine

Eserine was determined by a modification of a method described by Hellberg (1949a).

The sample (4·00 ml. containing about 8 mg. of eserine salicylate and 80 mg. of pilocarpine hydrochloride) is pipetted into a separating funnel. Sodium carbonate (M, 1 ml.) is added and the solutions rapidly extracted with peroxide-free ether (4 × 40 ml.). The ether phases are siphoned from the funnel, combined, dried with anhydrous sodium sulphate and filtered. Sulphuric acid (0·1N, 10·0 ml.) is added and the ether is evaporated under vacuum in a rotating evaporator in a water-bath at 35°. The remaining solution is transferred to a 25 ml. volumetric flask and water is added to the mark. 10·00 ml. is pipetted into a 50·0 ml. volumetric flask, sodium hydroxide (M, 1·9 ml.) is added, and after standing for 15 min. the mixture is gently shaken mechanically about 80 times/min. Potassium dihydrogen phosphate, (0·1M, 20 ml.) is then added, the solutions made up to the mark and the extinction determined at 500 mμ. The corresponding amount of eserine is read from a standard curve prepared by the same procedure. A straight line passing through origin is obtained within the concentration range of 1–4 mg./ml. of eserine salicylate.

Results and Discussion

A 0·2 per cent aqueous solution of pilocarpine hydrochloride showed no loss after 21 months in the dark and only 5 per cent loss after the same period in the light. The results of the other stability experiments are shown in Tables I and II. The optical rotation was unchanged after heating and storage for 12 months, indicating no transformation to isopilocarpine. Thus pilocarpine withstands heating at 110° for 30 min. and is stable for at least one year when stored at room temperature in the dark, even after being subjected to heat treatment.

TABLE I

STABILITY OF PILOCARPINE HYDROCHLORIDE AFTER HEATING AT 110° FOR 30 MIN.

Solution	Residual per cent pilocarpine hydrochloride		pH values of solutions			
	After storage for 12 months without previous heating	After heating and storage for 12 months	Before heating	Immediately after heating	After storage for 12 months without previous heating	After heating and storage for 12 months
Oculoguttae pilocarpini (Ph.S. Ed XI) stored in bottles ..	97·0	97·0	4·2	3·5	3·4	3·5
Oculoguttae pilocarpini (Ph.S. Ed XI) stored in ampoules ..	97·0	97·0	4·2	3·7	3·5	3·5

According to Riegelman and Vaughan (1958), a solution of eserine salicylate containing sodium bisulphite and a small amount of citric acid can be sterilised by autoclaving. This could not be verified in the present investigation. Heating to 120° for 20 min. caused 25 per cent loss of

eserine. The only possible method proved to be heating to 100° for 15 min. A preservative, e.g. phenylmercuric nitrate, should therefore be added. The sterility of the solution should be checked by a bacteriological test such as prescribed in the Swedish Pharmacopoeia for aseptically prepared solutions.

TABLE II

STABILITY OF PILOCARPINE AND ESERINE IN OPHTHALMIC SOLUTIONS STERILISED BY FILTRATION OR BY HEATING TO 100° FOR 15 MIN. AND STORED FOR 18 MONTHS UNDER DIFFERENT CONDITIONS

Storage conditions			pH values			Residual per cent eserine salicylate after 18 months	Residual per cent pilocarpine hydrochloride after 18 months
Temp. °C	Type of containers	Sterilising methods*	Before heating	Immediately after heating	After 18 months		
4–6	Bottle	A	—	—	3·4	98	101
		B	3·5	3·5	3·4	97	—
12–15	Ampoules	A	—	—	3·3	100	101
		B	3·5	3·5	3·3	100	102
	Bottle	A	—	—	3·5	101	—
		B	3·5	3·4	3·4	98	—
−10 to −30	Ampoules	A	—	—	3·2	100	103
		B	3·5	3·5	3·2	100	—
	Bottle	A	—	—	3·2	99	—
		B	3·5	3·4	3·2	100	101

A = filtration.
B = heating to 100° for 15 min.

As seen from Table II, the solution can be heated at 100° for 15 min. Stored in the containers described by Linde (1957), it is stable for at least 18 months even under greatly varying temperature conditions.

REFERENCES

Extra Pharmacopoeia, Martindale (1958), Vol. I, 24th ed. p. 1059. London: The Pharmaceutical Press.
Hellberg, H. (1949a). *Svensk Farm. Tidskr.*, **53**, 637–643.
Hellberg, H. (1949b). *Ibid.*, **53**, 658–663.
Levine, J. and Horrocks, E. (1960). *J. Assoc. off. agric. Chem., Wash.*, **43**, 233–234.
Linde, S. (1957). *Svensk Farm. Tidskr.*, **61**, 318–320.
Riegelman, S. and Vaughan, Jr., D. G. (1958). *J. Amer. pharm. Ass., Pract. Pharm. Ed.*, **19**, 474–477.
Schradie, J. and Miller, O. H. (1959). *Ibid.*, **20**, 197–199.

NEW APPARATUS

AN APPARATUS FOR THE LONG-TERM COLLECTION OF URINE FREE FROM FAECAL AND FOOD CONTAMINATION

BY R. T. BRITTAIN AND P. S. J. SPENCER

From the Research Division, Allen & Hanburys Limited, Ware, Herts.

Received January 28, 1963

An apparatus for the long-term collection of urine free from faecal and food contamination has been constructed using a galvanised wire cage suspended above a smooth glass plate inclined at an angle such that food and faeces are deflected from the urine collector.

DURING acute, sub-acute and chronic investigations of toxicity in small laboratory animals, it is often necessary to collect urine continuously for 24 hr. or longer to enable electrolyte, protein and urea excretion patterns to be determined. At the same time, it is essential that the animals should be maintained under normal laboratory conditions and allowed free access to food and water. The separation of urine from faeces by means of urino-faecal separators has been described by Harned, Cunningham and Gill (1949), Draper and Robins (1956), Brittain (1959) and others. However, the devices described are generally suitable for short-term experiments only and the animals are usually deprived of food and water. During the course of a sub-acute toxicity test in rats, an apparatus has been developed that is suitable for the long-term collection of urine, relatively free from contamination.

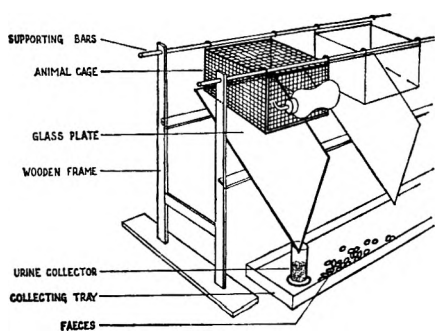


FIG. 1. Apparatus for the collection of urine free from food and faecal contamination.

The apparatus is shown in Fig. 1. A conventional galvanised wire mesh cage, dimensions 10 in. × 10 in. × 7 in., is suspended above a smooth glass plate inclined from front to back at 45°. The glass plate is 13 in. wide, the two parallel sides are 19 in. and 13 in. long respectively, whilst the diagonally cut side is about 14 in. long and has been ground smooth, rounded and annealed. Urine, food and faeces fall from the cage onto the glass plate, but because of the incline most of the food

and faecal matter rolls off the plate and is collected underneath the apparatus in a flat metal tray. Urine runs down the glass plate until it reaches the lower edge and because of a second incline on this edge, flows along it towards the glass container. Bacterial growth in the urine is prevented by the prior addition of 0·2 ml. of a 1 per cent thiomersal solution. Occasionally, faecal pellets find their way into the collecting vessel, but this can be avoided by making the glass plate slightly oversize and placing the collecting vessel out of direct line to the cage. Because of the shape and size of the apparatus, it is convenient to use 4 or 5 cages and separators together in a battery. A wooden frame at each end of the battery carries metal rods which pass through and support the cages; the glass plates are attached by screws to wooden bearers also running the length of the battery. In this manner, 5 cages and separators can be accommodated in a space 6 ft. by 3 ft.

The cages described are suitable for groups of 4 rats (up to 150 g. body weight). Larger groups would obviously require a larger cage, but the design of the apparatus is adaptable for this. Food is presented as a paste (equal parts food and water) in a metal box attached to the inside of the cage; water is allowed *ad libitum*. In the average 24 hr. collection of urine, no more than a dozen faecal pellets and about 2 per cent of the initial food adhere to the glass plate. If the plate is scraped once or twice each day, the chance of this residue absorbing urine is minimised. It is estimated that approximately 80 per cent of the excreted urine is collected, and this is facilitated by washing the glass plate daily with a 10 per cent solution of a wetting agent (Teepol) and allowing this to dry on the plate.

A disadvantage of this apparatus for separating urine from food and faeces is the large surface area of glass from which evaporation readily occurs. However, by maintaining the temperature of the room at 70° F and ensuring absence of draughts, evaporation losses can be kept to a minimum. Quantitative recovery of urinary constituents left behind on the glass can be effected by washing down the plate with distilled water, and combining the urine and water fractions.

TABLE I

EFFECT OF URANYL ACETATE ON THE EXCRETION OF UREA, PROTEIN AND WATER BY INTACT RATS

Group	Index of kidney function	Day of experiment								
		−6	−3	0	1	2	3	4	11	18
A (Normal saline)	Urine volume, ml./24 hr.	19	19	14	18·5	25	20·5	24	18	18
	Urea g./24 hr.	0·57	0·61	0·50	0·71	0·59	0·63	0·43	0·64	0·80
	Total protein mg./24 hr.	29·9	28·5	23·5	37·7	21·0	38·5	20·2	29·4	22·3
B (Uranyl acetate 5 mg./kg.)	Urine volume ml./24 hr.	21	14	18	20	36	44	44	43	23
	Urea g./24 hr.	0·63	0·58	0·74	0·26	0·05	0·11	0·11	0·58	0·41
	Total protein mg./24 hr.	31·5	22·1	27·8	50·0	56·2	172·5	161·3	150·9	49·7

Table I summarises the results from a typical experiment using this apparatus. Two groups, A and B, each of 4 male rats of initial body

weight 120–130 g., were maintained in the apparatus for several days to obtain urine samples before drug treatment. Group A then received normal saline and group B 5 mg./kg. uranyl acetate subcutaneously, the dose volume injected being 1·0 ml./kg. Cameron, Burgess and Trenwith (1947) have reported that uranyl acetate has a specific damaging effect on the kidney. The results of this experiment show that uranyl acetate causes an immediate fall in urea excretion and an increase in urinary volume (indicating a loss of tubular function) and a marked proteinuria (indicating breakdown of kidney tissue). Because this apparatus allows free access to food and water throughout the period of collection, changes in urinary constituents cannot be due to changes in diet, but to uranyl acetate-induced changes in kidney function.

Using groups of 4 or 5 rats the apparatus has been found to effectively collect urine relatively free from faecal and food contamination. Furthermore this collection of urine can be achieved without transferring animals from their stock cages. This is desirable because animals should not be subjected to a change of environment during sub-acute or chronic toxicity tests. The apparatus is simple, cheap, compact, easy to clean and easily reproducible for experiments using larger numbers of animals.

References

Brittain, R. T. (1959). *Laboratory Practice*, **8**, 279.
Cameron, G. R., Burgess, F. and Trenwith, V. S. (1947). *Brit. J. Pharmacol.*, **2**, 59–64.
Draper, H. H. and Robins, A. F. (1956). *Proc. Soc. exp. Biol., N.Y.*, **91**, 174–175.
Harned, B. K., Cunningham, R. W. and Gill, E. R. (1949). *Science*, **109**, 489–490.

LETTERS TO THE EDITOR

Effect of Hydrogen Peroxide on the Colour of the Fluorescence of Oestrone in Concentrated Sulphuric Acid

SIR,—Oestrogens in solution in concentrated sulphuric acid when irradiated with ultra-violet light have fluorescent intensities which differ sufficiently to allow the detection and differentiation of oestrone, oestradiol and oestriol from one another on paper chromatograms and for their quantitative assay (Ittrich, 1960; Preedy and Aitken, 1961). When dissolved in amounts of approximately 1 μg./ml. of sulphuric acid, oestrone fluoresces green-yellow; oestradiol, gold-yellow, and oestriol, orange-yellow.

The addition of small amounts of 30 per cent aqueous hydrogen peroxide to such solutions affects their ability to fluoresce: oestrone emits a blue fluorescence of high intensity, whereas with oestriol and oestradiol, the fluorescence is quenched.

The blue fluorescence of oestrone was used to determine its presence when in admixture with oestradiol and oestriol. A Pulfrich photometer with ultra-violet equipment was used with a C comparator plate as a standard light source.

From an ethanolic (95 per cent w/v) solution of oestrone (10 μg./ml.), appropriate amounts are transferred to Jena-glass test-tubes of inner diameter 8 mm. The ethanol is evaporated and each dry residue dissolved in 93·5 per cent sulphuric acid (1 ml.). This is followed immediately by the addition of 30 per cent hydrogen peroxide (0·05 ml.). Transference of the tubes to the photometer and measurement of the intensity of the blue fluorescence must be made after 5 min. and at a temperature of 22°. These are the optimal conditions for the determination. The intensity of the blue fluorescence increases for a short time after the addition of hydrogen peroxide and then decreases; it varies with the concentration of sulphuric acid used, the amount of peroxide added and the temperature. Hence, it is necessary to adhere strictly to the conditions of the procedure.

From a graph, plotting the intensity of the blue fluorescence against the amount of oestrone, a linear relation was found over the range of 10–40 μg. of the drug.

Oestrone can be determined quantitatively in this way even though the apparatus used has a relatively low sensitivity. The fluorescence of oestrone solutions in sulphuric acid without the addition of hydrogen peroxide is too weak to make this quantitative determination possible.

ALEKSANDRA SMOCZKIEWICZOWA
ROMAN SIODA

Institute of Inorganic and Analytical Chemistry,
Medical Academy,
Poznań, Poland.
May 8, 1963

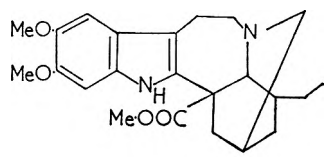
REFERENCES

Ittrich, G. (1960). *Hoppe Seylers Z. Physiol. Chem.*, **320**, 103.
Preedy, J. R. K. and Aitken, E. H. (1961). *J. biol. Chem.*, **236**, 1297–1299.

The Isolation and Identification of the Major Alkaloid Present in *Tabernaemontana pachysiphon* Stapf. var *cumminsi* (Stapf.) H. Huber

SIR,—We have isolated and identified an alkaloid from *T. pachysiphon* var *cumminsi*. The plant was collected from the Mpraiso district, Ghana, the leaves dried below 50° and the alkaloids extracted by percolation with ethanol. A soft extract was prepared by distillation under reduced pressure. It was dissolved in glacial acetic acid and poured into a large volume of water with vigorous stirring. The aqueous acidic layer was separated from the tars and the total bases precipitated with ammonia in the presence of ice. The major alkaloid was separated from the mixed bases by chromatography on alumina using benzene (or chloroform), purified by preparing the tartrate salt which was recrystallised from methanol m.p. 220°. The free base was isolated and recrystallised from methanol m.p. 144–145°. Analysis gave an empirical formula $C_{23}H_{30}N_2O_4$. Ultra-violet spectrum in absolute ethanol gave λ_{max} 223 mμ (ϵ = 15,600) and λ_{max} 302 mμ (ϵ = 6,550). The spectrum was unchanged in acidic and strongly basic conditions. The major infra-red bands were at 1725 cm.$^{-1}$ and (ester carbonyl) and at 3,350 cm.$^{-1}$ (N–H-stretching) in Nujol mull.

A mass spectrometric examination of the alkaloid was carried out and gave the data consistent with that reported by Biemann (1962) on a series of iboga alkaloids and was suggestive of the iboga alkaloid conopharyngine (I) which



[I]

has been isolated by Renner, Prins and Stoll (1959) from *Conopharyngia durissima* (Stapf.). The m.p. ultra-violet and infra-red spectra are in good agreement with those of Renner, Prins and Stoll (1959). A mixed m.p. with an authentic sample of conopharyngine showed no depression. Smaller quantities of minor alkaloids have been obtained by chromatography on alumina and these will be reported on in due course.

Acknowledgments. The authors wish to thank F. N. Hepper, Royal Botanic Garden, Kew, for botanical verification, Dr. A. W. Bowen of "Shell" Research Limited for the mass spectrum and Geigy Ltd. for supplying a reference sample of conopharyngingine.

J. THOMAS

Department of Pharmacy,
University of Manchester,
Manchester, 13.

G. A. STARMER

Department of Pharmacy,
Kwame Nkrumah University,
Kumasi, Ghana.
March 22, 1963

REFERENCES

Biemann, K. (1962). *Mass Spectrometry*. New York: McGraw-Hill.
Renner, U., Prins, D.A. and Stoll, W.G. (1959). *Helv. Chim. Acta.*, **42**, 1572–1581.

A Rapid Turbidimetric Method for Heparin Assay

SIR,—During an investigation of the column chromatographic behaviour of several polyanions we felt the need for a procedure for heparin determination which would enable us to deal rapidly and inexpensively with a large number of samples.

Methods already published (Jaques and Bell, 1959) did not meet our requirements and we were compelled to develop a new procedure which might interest other workers in this field.

It is known that the basic antibiotic, streptomycin, forms water-insoluble complexes with high molecular weight polyphosphates (Cohen and Lichtenstein, 1960; Harshaw, Brown and Graham, 1962). Although the molecular weight of heparin is well below the critical size required for the polyanions' reaction with streptomycin, it was thought that the greater degree of ionisation of the heparin-like polysulphates would nevertheless allow the reaction to occur. This was indeed so. In addition, the turbidity produced by the insoluble heparin-streptomycin complex obeys the Beer's relationship within certain concentration limits. This finding served as a basis for the procedure for heparin assay outlined below.

Mix 1·0 ml. of sample containing 15–300 μg./ml. of heparin with 3·0 ml. 0·25 per cent streptomycin sulphate (NBC, diagnostic). Dihydrostreptomycin of reagent grade may also be used. Allow to stand 10 min. at 18–22° and read the extinction in a Pulfrich step photometer with the L_2 filter (maximum transmittance at 480 mμ). If a spectrophotometer is available, readings are preferably taken at 400 mμ against a suitable blank. The turbidity is stable for at least 120 min. at 18–22°. The standard solution (containing 100 μg./ml.) is stabilized by dissolving 10 mg. of sodium heparin (BDH) in 100 ml. of 2 per cent benzoic acid.

The relation between extinction and concentration is linear over the range 0–300 μg. heparin per ml. The standard deviation computed from a series of 10 parallel determinations on the same solution is below 0·01 (Beckman DU spectrophotometer) or 0·03 (Pulfrich step photometer). The method is non-specific, a defect shared by other chemical methods for the determination of heparin.

TABLE I

EFFECT OF IONIC STRENGTH ON THE TURBIDITY PRODUCED BY THE INTERACTION OF HEPARIN WITH STREPTOMYCIN

The reaction mixture contained in 4·0 ml. final volume 200 μg. heparin, 0·0–0·5M halide and 10 mg. streptomycin sulphate. The figures represent the decrease in extinction as per cent of the extinction of the tube containing no halide = 100 per cent. The ionic strength is computed from the formula: $I = 0{\cdot}5\ \Sigma\ [i].Z_i^2$.

Ionic strength	LiCl	NaCl	KCl	$MgCl_2$	$MnCl_2$
0·025	93	98	91	—	—
0·05	89	89	83	83	78
0·10	79	81	78	78	76
0·15	75	70	65	—	—
9·20	64	64	60	67	62
0·25	56	54	55	54	50

All other polyanions so far tested, except some commercial samples of yeast ribonucleic acid gave similar reactions with streptomycin. In this connection it is interesting to note that another basic antibiotic, neomycin sulphate, is

capable of precipitating relatively small molecules of ribonucleic acid of the "soluble" type.

The reaction between heparin and streptomycin is sensitive to the ionic strength of the medium. The interference of some representative halides with this reaction is presented in Table I. It is reasonable to assume that the neutralisation of the sulphate groups of heparin by the guanidine residues in the streptomycin molecule leads to reversible breaking of the hydrogen bonds between the polyanion and water. It thus seems that the interaction between soluble polyelectrolytes does not lead to the formation of firm covalent bonded complexes. Therefore, it is essential to desalt the assayed sample before the addition of streptomycin, and to prepare the latter reagent with deionised water.

Laboratory Division,
General Hospital of Gaesti, Arges,
P.O. Box 204, Bucharest 1,
Rumania.

E. J. ALTESCU

April 24, 1963

REFERENCES

Cohen, S. S. and Lichenstein, J. (1960). *J. biol. Chem.*, 235, PC55.
Harshaw, S. P., Brown, R. A. and Graham, A. F. (1962). *Analyt. Biochem.*, 4, 182.
Jaques, H. B. and Bell, H. J. (1959). In *Methods of Biochemical Analysis*. VII, 292. Editor Glick, D. New York: Academic Press.

Colomycin and Polymixin E

SIR,—During investigations upon the mode of action of the polypeptide antibiotic colomycin and using *Escherichia coli* we were impressed by the close similarity between colomycin and polymixin E as judged by the effect of inoculum size on minimum inhibitory concentration, the adsorption of the antibiotics from aqueous solution by washed suspensions of the test organism, and the pattern of leakage of cellular material absorbing at 260 mμ.

Subsequent investigations showed that the two samples possessed indistinguishable infra-red spectra when examined in KBr discs. The effects of varying the concentrations of each antibiotic upon the surface tension of water were identical as measured by the De Nouy tensiometer.

R_F values using paper chromatography and two solvent systems are shown in Table I.

TABLE I

R_F VALUES OF COLOMYCIN AND POLYMIXIN E USING TWO SOLVENT SYSTEMS

Solvent system	R_F	
	Colomycin	Polymixin E
n-Butanol : glacial acetic acid : water 4 : 1 : 5	0·398	0·397
n-Butanol : glacial acetic acid : acetone : water 2 : 0·5 : 2·1 : 0·1	0·75	0·74

A mixture of colomycin and polymixin E ran as one spot using the same solvent systems. Both samples were hydrolysed with 5N hydrochloric acid

(6 hr. at 125°). The hydrolysates were subjected to ion-exchange chromatography by the method of Moore and Stein (1951) but using Zeo-carb 215. The percentage content of threonine, leucine and 1,3-diaminobutyric acid obtained by this method were as shown in Table II.

TABLE II

PERCENTAGE CONTENT OF THREE AMINO-ACIDS IN HYDROLYSATES OF COLOMYCIN AND POLYMIXIN

	Threonine	Leucine	1,3-Diaminobutyric acid
Colomycin	18·6	25·3	56·1
Polymixin E	20·7	22·7	56·5

We feel that these data, although obtained with colomycin from a different source, might be of interest in view of the findings of Wilkinson (1963).

We thank Pharmax Ltd. for a gift of colomycin and Burroughs Wellcome and Co. for a gift of polymixin E.

Department of Pharmacy,
The University,
Nottingham.

W. B. HUGO
R. J. STRETTON

May 20, 1963

REFERENCES

Moore, S. and Stein, W. H. (1951). *J. Biol. Chem.*, **192**, 663–681.
Wilkinson, S. (1963). *Lancet* (i), 922–923.

BOOK REVIEWS

PHARMACOGNOSY OF AYURVEDIC DRUGS (Kerala). Series 1, No. 5. By K. Narayana Aiyar and M. Kolammal. Pp. vi + 123. Department of Pharmacognosy, University of Kerala, Trivandrum, 1962. Rs.10.

This book is No. 5 of a treatise describing the Ayurvedic drugs of India, especially those used in the province of Kerala (Travancore) in Southern India. The "Ayurveda' is an ancient Sanskrit text describing a system of medicine and the word means the "Science of Life." No. 5 contains a description of nine drugs arranged under their ayurvedic names. The nine drugs are derived from 22 plants. indicating the difficulty of attaching each name to a single plant. It is obvious, therefore, how necessary it is to publish the descriptions given in this book, thus providing a means of establishing their sources by accurate descriptions and excellent drawings, several of which are coloured, of the plants, as well as an account of the anatomy, accompanied by careful drawings, of the parts which are used medicinally in India. Each drug is described under two headings, first, the ayurvedic name followed by quotations in Sanskrit which is transliterated into arabic lettering; this is followed by a list of the many terms applied to its parts, together with the meaning in English of each term. The second heading is the modern botanical name beneath which is a careful description of the plant and then an account of the histology of those parts used medicinally. For the anatomical drawings the same abbreviations are used throughout the book and are listed in the introducion, thus avoiding repetition in the legend to each set of drawings.

Of the drugs described some are well known in European medicine:—

Kapikacchu from *Mucuna prurita* is usually named cowhage or itching powder and consists of the hairs of the fruit epidermis and used as a vermifuge for round-worm and thread-worm. Other parts are used medicially in India, viz. the roots and seeds.

Ksiravidari from *Ipomoea paniculata* (= *Batatas edulis*) yields the sweet potato, the starch of which is sometimes named Brazilian Arrowroot and may appear as a substitute or adulterant for some of the other starches used in European medicine and food.

Jambu from *Syzygium jambolanum* (= *Eugenia jambolana*) yields seeds, known in Europe as Jambool seeds and are said to be useful in diabetes. In addition the bark and fruits are used in India and the bark is fully described and its anatomy with drawings of details.

Kiratatiktah from *Andrographis paniculata* is well known and has been described in European literature as an adulterant or substitute for Chiretta and is used as a bitter tonic.

The book forms a valuable contribution to the knowledge of indigenous Indian medicinal plants and will help to encourage investigation of the very numerous herbal medicines so largely used in the Indian sub-continent.

T. E. WALLIS.

PHARMACOLOGY AND THERAPEUTICS. Fifth, revised and enlarged, edition. By Arthur Grollman. Pp. 1131 (including Index and 236 illustrations). Henry Kimpton, London, 1962. 93s. 6d.

The appearance of the 5th Edition of Grollman's *Pharmacology and Therapeutics* coming as it does only two years after the 4th Edition and eleven years after the first, is a sure sign of a successful textbook. By American standards this is a medium-sized or even smallish book. Like earlier editions it is readable, lucid, well printed and well bound.

It is however very much a textbook of pharmacology and therapeutics aimed primarily at the undergraduate in medicine or the medical practitioner rather than a book which deals with the scientific basis of pharmacology. This is perhaps its greatest weakness as a text. On the other hand it is an up-to-date volume and deals with a very wide range of modern drugs and preparations, some of which are unfamiliar to the British reader.

Each chapter is supplied with a useful list of references for further reading, and where appropriate, with a list of relevant U.S.P. preparations. It is sad to see in this of all textbooks, that B.P. preparations are no longer included. The author, like all who write texts in pharmacology, has been faced with the dilemma of what to retain and what to leave out; thus relatively large sections are devoted to the bromides and cocaine whilst compounds such as halothane and the thiazide diuretics deserve more extensive treatment than they get. Nicotine is dealt with at length, yet suxamethonium receives only brief attention. Two pages are devoted to the Veratrum alkaloids which is more than the total space devoted to mecamylamine and guanethidine, and one would have liked to read more about griseofulvin. The book is undoubtedly very good in dealing with drug side effects, and there are a number of interesting and informative illustrations of these. Oddly enough, no mention is made of the side effects associated with thalidomide.

The chemical formulae shows some inconsistencies and the book would be improved if they were all re-drawn by the same hand and the formulae revised by an organic chemist. It is however a good book which repays reading. Perhaps its greatest value lies in the easy accessibility of a great deal of information—there is no need to cope with irrelevancies or repetition because these hardly exist. It will be welcomed by most undergraduates with a leaning towards the clinical applications of pharmacology. Others will find it of the greatest value when supplemented by the texts dealing with the scientific aspects of the subject.

J. J. LEWIS

PRINTED BY W. HEFFER & SONS LTD., CAMBRIDGE, ENGLAND.

General Medical Council

BRITISH PHARMACOPŒIA 1963

THE BRITISH PHARMACOPŒIA is published under the direction of the General Medical Council in accordance with the terms of the Medical Act, 1956 and provides standards for the quality of substances and preparations used in medical and pharmaceutical practice. New editions of the Pharmacopœia are published at intervals of about five years. The British Pharmacopœia first appeared in 1864 and the British Pharmacopœia 1963 thus constitutes the centenary edition.

The new edition contains almost 1000 monographs, of which more than 200 appear for the first time. All the monographs and appendices retained from the previous edition of 1958 have been thoroughly revised and many modifications made in the specifications and methods of test.

Monographs on new drugs and preparations include antibiotics, steroids, hypnotics, sulphonamides, hypertensive agents, antihistamines, tranquillisers, diagnostic agents and immunological products.

The Appendices have been extended to 278 pages and include specifications for reagent chemicals, physical, chemical and biological methods of assay and test, and a section on the design and precision of biological assays.

Publication date July 1, 1963; official in the United Kingdom from January 1, 1964.

Pp. xxviii + 1210. Price £5 (plus 2s. 6d. U.K. postage).

Published for the General Medical Council by The Pharmaceutical Press.

Pharmaceutical Society of Great Britain

British Pharmaceutical Codex 1963

THE BRITISH PHARMACEUTICAL CODEX is an international reference work providing up-to-date information on drugs and drug formulations, immunological products, human-blood products, surgical ligatures and sutures, and surgical dressings.

The Codex has a dual role. It gives authoritative and up-to-date information on nearly 1000 medicinal and related substances in current use. And it also provides *standards* for many substances in current use that are not included in the British Pharmacopœia.

Information on the *actions and uses of drugs* is prepared by a panel of medical and pharmaceutical experts after thorough discussion of published evidence and personal experience.

The 1963 Codex includes monographs on more than 120 new drugs, and on a number of new materials used in formulation.

Other subjects of new monographs include sutures made of stainless steel and of various polyesters, plastic first-aid dressings, and *x*-ray detectable surgical swabs. For the first time the Codex describes the *uses* of surgical dressings.

Nearly 70 new formulæ are included, and significant changes have been made in some 30 preparations. In particular there are important changes in the monographs on eye drops.

Publication date July 1, 1963; official in the United Kingdom from January 1, 1964.

Pp. xxxvi + 1432. Price £5 5s. (plus 2s. 9d. U.K. postage).

Published by The Pharmaceutical Press.

The Pharmaceutical Press
17 Bloomsbury Square, London, W.C.1